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ARTICLE

Clupeid Response to Stressors: The Influence of Environmental Factors on Thiaminase Expression

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Abstract

Over the past five decades, a reproductive failure related to thiamine deficiency, referred to as thiamine deficiency complex (TDC), has been observed in valuable salmonine fishes in the Great Lakes and Finger Lakes in North America and the Baltic Sea in Europe. The cause of TDC has been linked to the consumption of clupeid fish, which contain high levels of a thiamine-destroying enzyme called thiaminase I (hereafter referred to as "thiaminase"). High activities of thiaminase have been reported from clupeids such as Alewife *Alosa pseudoharengus*, Gizzard Shad *Dorosoma cepedianum* and Atlantic (Baltic) Herring *Clupea harengus*, but no consistent explanation has accounted for the wide range of observed variation in levels of thiaminase in clupeids. Chronic stress can suppress the immune systems of Alewife and other fishes, thereby reducing the number of circulating white blood cells available to suppress bacteria. Because the presence of thiaminase has been associated with thiaminolytic bacteria isolated from Alewife viscera, we hypothesized that stressful conditions, which can potentially limit clupeid immune response or alter internal physiological conditions, could allow for thiaminase to be produced more efficiently by bacteria or thiaminolytic bacteria could proliferate, or both events could occur, resulting in a subsequent increase in thiaminolytic activity. In this study, Alewives and Gizzard Shad were exposed to severe winter temperatures and low food availability, respectively, in replicated pond experiments to evaluate the influence of stressful conditions on clupeid thiaminase activity. Though responses in circulating white blood cell counts and metrics of fish condition indicated that experimental treatments affected these clupeids, these effects were not related to increased thiaminase activity. The only significant treatment effect on clupeid thiaminase was an increase in mean thiaminase activity in Gizzard Shad from ponds where only high quality energy sources were available. These data indicate that variability in clupeid thiaminase may be related to diet composition.

Reproductive failure in piscivorous salmonine fishes has been observed over the past five decades in the Laurentian Great Lakes and Finger Lakes in North America and the Baltic Sea in Europe. This phenomenon was first observed in the broodstocks of salmonine fish in North American hatcheries during the 1960s (McDonald et al. 1998), and reproductive failure in Baltic Sea salmon became prevalent in the 1970s (Hansson et al. 2001). Mortality of Coho Salmon *Oncorhynchus kisutch* fry that exhibited similar symptoms was first observed in wild fish in 1967 (Johnson and Pecor 1969). During the next several decades managers and researchers working in the Laurentian Great Lakes and Finger Lakes of New York recognized that other salmonine fishes, including Lake Trout *Salvelinus namaycush*, Atlantic Salmon *Salmo salar*, Chinook Salmon *O. tshawytscha*, Rainbow Trout *O. mykiss*, and Brown Trout *S. trutta*, suffered from a similar reproductive failure (Fisher et al. 1995; Marcquenski and Brown 1997; McDonald et al. 1998).

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In North America the syndrome associated with this type of reproductive failure has been referred to as thiamine deficiency complex (TDC), which has been linked to thiamine deficiency (Fitzsimons et al. 1999). During the mid-1990s it became increasingly evident that thiamine-deficient salmonids were susceptible to TDC (Fisher et al. 1996; Fitzsimons et al. 1999). Thiamine (vitamin B_1) is an essential vitamin necessary for the conversion of carbohydrates and lipids into energy and is ubiquitous for sustaining organisms across all kingdoms of life. Offspring of salmonine fishes susceptible to TDC die shortly after hatching, but fry from identical egg sources survive and exhibit normal behavior when treated with thiamine (Fitzsimons 1995; Fisher et al. 1996).

Clupeid fishes, including Alewife *Alosa pseudoharengus*, Gizzard Shad *Dorosoma cepedianum*, and Atlantic (Baltic) Herring *Clupea harengus*, have been routinely observed to contain high activity levels of thiaminase I, a thiamine-destroying enzyme (Wistbacka et al. 2002; Tillitt et al. 2005). A large body of field and laboratory work conducted during the past two decades supports a plausible hypothesis for an association between a prey base composed of a large biomass of clupeids containing thiaminase I and the recruitment failures often experienced by piscivorous salmonines. For example, observations of sac-fry mortality in Atlantic Salmon (Norrgren et al. 1993; Bengtsson et al. 1994; Karlsson et al. 1996) and sea-run Brown Trout (Soivio 1996) in the Baltic Sea were attributed to the consumption of clupeid prey containing thiaminase I. Although two thiamine-degrading enzymes have been described (thiaminase I and II; see Discussion for more about thiaminase II), thiaminase I has been the specific thiaminase linked to reproductive failure in fish; therefore, all subsequent references to thiaminase in this paper specifically refer to thiaminase I.

Thiaminase activity in Alewives has been attributed to thiaminase positive bacteria *Paenibacillus thiaminolyticus* isolated from Alewife viscera (Honeyfield et al. 2002), though Richter et al. (2012) reported that this bacteria is not the primary source of thiaminase activity. Thus, there is some debate over whether the ultimate source of thiaminase in fish is the fish themselves (Riley and Evans 2008; Richter et al. 2012), thiaminolytic bacteria (Honeyfield et al. 2002), other sources, or a combination of these sources. Nevertheless, the presence of thiaminase in Alewives is believed to be a primary factor responsible for TDC in predators of the Alewife (Fitzsimons et al. 1999; Honeyfield et al. 2005a) even though thiamine deficiency has not been observed within clupeids containing high thiaminase activity (Wistbacka et al. 2002; Tillitt et al. 2005). Laboratory experiments have induced TDC in Lake Trout by altering dietary levels of thiaminase, using feral Alewives containing thiaminase and bacterial sources of thiaminase (Honeyfield et al. 2005b). Although the ultimate source or sources of thiaminase contributing to TDC are unknown in natural systems (Richter et al. 2012), available evidence suggests that clupeid or bacterial thiaminase, or both, play a key role in the development of salmonine thiamine deficiency.

Both TDC in salmonines and thiaminase activity in clupeids fluctuate widely, and a mechanistic understanding of the processes influencing thiaminase activity in clupeids remains unknown (Wistbacka et al. 2002; Brown et al. 2005; Fitzsimons et al. 2005; Tillitt et al. 2005; Ikonen 2006). However, if internal microbial populations are a source of thiaminase activity in the Alewife and other clupeids, changes in physiological conditions (e.g., those resulting from environmental or other postulated sources of stress) that influence the growth characteristics of bacteria can be expected to affect thiaminase activity (Tillitt et al. 2005). For example, increases in Alewife thiaminase activity have been observed in association with decreases in water temperature in natural systems and transport by truck and subsequent holding in captivity (Tillitt et al. 2005; Lepak et al. 2008; J. Fitzsimons, Fisheries and Oceans Canada, personal communication), and with immune system challenge (Wistbacka et al. 2009). These increases in thiaminase activity may be related to physiological stress, though the mechanism behind the observed increases remains unknown.

Despite the fundamental role of clupeids in producing TDC in valuable apex predators including sport fishes, little is known about the factors responsible for inducing thiaminase production in clupeids. Although research has focused mainly on Alewife and Baltic Herring, other clupeid fishes can contain thiaminase in high concentrations. For example, Gizzard Shad have high levels of thiaminase activity (means from 15,000 to 30,000 pmol thiamine$\cdot g^{-1}\cdot min^{-1}$: Tillitt et al. 2005; Honeyfield et al. 2008) relative to that for Alewife (on the order of 5,000 pmol thiamine$\cdot g^{-1}\cdot min^{-1}$: Tillitt et al. 2005). Given the tendency of Gizzard Shad to dominate biomass and their wide distribution in eastern U.S. waters (Bachmann et al. 1996; Hale et al. 2008), thiaminase in Gizzard Shad has the potential to negatively affect apex predator populations, the diets of which are dominated by these prey fish. Therefore, we designed a set of experiments in replicated pond systems to evaluate the influence of stressful conditions on the thiaminase activity in Alewives and Gizzard Shad. Given the potential association between thiaminolytic bacteria and thiaminase in Alewife (or the production of thiaminase by fish themselves), we expected that environmental stressors that can influence physiological characteristics of clupeids could also influence their thiaminase activity. Increased stress in clupeids could increase thiaminase activity through an indirect mechanism whereby thiaminase is produced more efficiently by thiaminolytic bacteria or the bacteria proliferate because of alterations in clupeid immune system function, or both, or thiaminase activity is increased directly by the induction of physiological changes in clupeids that result in the increased production of thiaminase. Severe winter temperatures and low food availability were selected as treatments in this set of experiments. These treatments were selected because Alewives can be sensitive to low temperatures (Colby 1973) and Gizzard Shad, in more oligotrophic systems (i.e., where food availability is low), tend to have low densities, growth rates, and condition factors (Bachmann et al. 1996; Hale et al. 2008). We hypothesized that

these treatments would result in stressful conditions, ultimately leading to an increase in clupeid thiaminase activity.

METHODS

Alewife pond experiment.—Alewives were collected from Waneta Lake, Schuyler County, NewYork, on the evening of 26 October 2004 and transported by truck to the Cornell Experimental Pond Facility in Ithaca, New York. Alewives (120–140 mm) were stocked at a density of 160 individuals into each of four fishless ponds (640 Alewives total) on the morning of 27 October 2004. Each individual pond was approximately 1,800 m^3 with a maximum depth of approximately 2 m. Contrasting winter temperatures were produced by maintaining ice-free conditions (using an aeration system described in Lepak and Kraft 2008) in two of the four study ponds from 11 December 2004 to 5 January 2005. On 21 April 2005 each pond was drawn down to a depth of approximately 1.0 m. A bag seine approximately 40 m long and 1.2 m tall was used to collect the remaining Alewives from the ponds. Alewives (10 from each pond) were collected for thiaminase analysis and analyses of circulating white blood cells. The remaining surviving Alewives from each pond were used for wet–dry weight analysis as an indicator of fish condition. Alewives collected for thiaminase analyses were flash frozen and stored at −80°C until analysis. Thiaminase analyses were conducted at the Canada Centre for Inland Waters, Burlington, Ontario, using the procedure described by Zajicek et al. (2005). Mixed-model analysis (pond as a random effect and treatment as a fixed effect using the PROC MIX procedure) and ANOVA were conducted using SAS (SAS 2010) to test for the effect of treatment on Alewife thiaminase and circulating white blood cell counts, and pond temperatures, respectively.

Gizzard Shad pond experiment.—This experiment was conducted at the experimental pond facility at the Ecology Research Center at Miami University, Oxford, Ohio. Replicate ponds (approximately 800 m^3 with a maximum depth of approximately 2.5 m) lined with heavy-duty plastic (covering any sediment present) were used to evaluate the influence of nutrient and sediment additions on pond chemistry and Gizzard Shad. Gizzard Shad from Acton Lake, Ohio, (source population) were stocked within the ponds. The treatments, each with three replicates, were: no nutrient or sediment addition (no additions), nutrient addition (+N), sediment addition (+S), and addition of nutrients and sediment (+N+S). The experiment was initially designed to evaluate the influence of agricultural sediment and nutrient inputs on Gizzard Shad (Pilati et al. 2009). However, a thiaminase component was added to evaluate the influence of stressful conditions associated with the lack of nutrients and sediments (a potential food source for Gizzard Shad: Babler et al. 2011) in ponds with no nutrient or sediment additions, relative to ponds in which nutrients, sediments, or both were added.

Details of the experimental set up and treatment conditions are available in Pilati et al. (2009). Briefly, all nutrient treatments (+N and +N+S) received weekly additions of ammonium nitrate (NH_4NO_3, to provide N) and sodium phosphate ($NaPO_4 \cdot H_2O$, to provide P) at loading rates of 150 μg N/L and 15 μg P/L in pond water per week. These loading rates, designed to stimulate phytoplankton production, are similar to those in Acton Lake (Vanni et al. 2001). The +S and +N+S treatments first received a ∼2–3 cm layer of sediments, and then weekly additions of sediments (0.06 m^3, ∼70 kg) dispersed evenly by means of a pump. Sediments were obtained near the inflows to Acton Lake, and hence presumably were composed mostly of allochthonous material. Weekly inputs mimicked the long-term sedimentation rate in Acton Lake (Renwick et al. 2005).

Ponds were filled on 29 May 2004 with water from an oligotrophic supply pond (6.8 μg chlorophyll/L) and each experimental pond was subsequently inoculated with 400 L of water from Acton Lake to supply plankton for colonization. On 10 June 2004, all ponds were stocked with 100 Gizzard Shad individuals, at a biomass of ∼175 kg wet mass/ha. Two size-classes of Gizzard Shad were stocked, juveniles (120–140 mm, 40 fish/pond) and adults (180–200 mm, 60 fish/pond). The experiment lasted 11 weeks, and on 8 September 2004, the ponds were drained and all adult Gizzard Shad were collected, weighed and measured, and Fulton's condition factor, K (an indicator of condition; $K = W/L^3 \times 100{,}000$, where W is the mass in grams and L is the length in mm: Williams 2000), was calculated.

Adult Gizzard Shad (three from each individual pond, nine in total for each treatment) were collected and frozen on dry ice for thiaminase analysis at two different times; the first group of fish were collected and sent to the laboratory for analysis within 5 d of collection, and the second batch was evaluated after storage at −20°C for approximately 9 months. Thiaminase analyses were conducted at the Canada Centre for Inland Waters, Burlington, Ontario, using the procedure described by Zajicek et al. (2005). ANOVA and ANCOVA were conducted using SAS (SAS 2010) to test for the effect of treatment on thiaminase activity and condition (Fulton's K), the relationship between thiaminase activity and condition (Fulton's K), and N and P excretion rates of in Gizzard Shad. However, due to the limited amount of data available for Gizzard Shad thiaminase, a Monte Carlo randomization simulation using 10,000 iterations was conducted using SAS (SAS 2010) to assess the validity of the relationship between thiaminase activity and condition in Gizzard Shad. Fulton's K was based on mean values for adult shad collected from each of the four treatments after the completion of the experiment.

RESULTS

Alewife Pond Experiment

Mean thiaminase activity in Alewives was not influenced by the treatments in this experiment. The treatments resulted in the fish being exposed for approximately 2 months (mid-December to mid-February) during which time the water

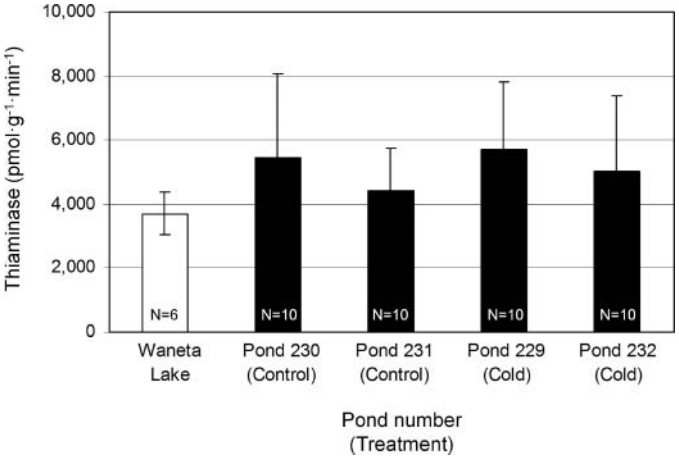

FIGURE 1. Thiaminase activities (mean ± SE) (pmol thiamine·g^{-1}·min^{-1}, denoted as pmol·g^{-1}·min^{-1}) in Alewives from the source population (open bar, Waneta Lake) and control and treatment experimental ponds (black bars). The number of fish tested (*N*) to establish each mean is shown at the base of the individual bars.

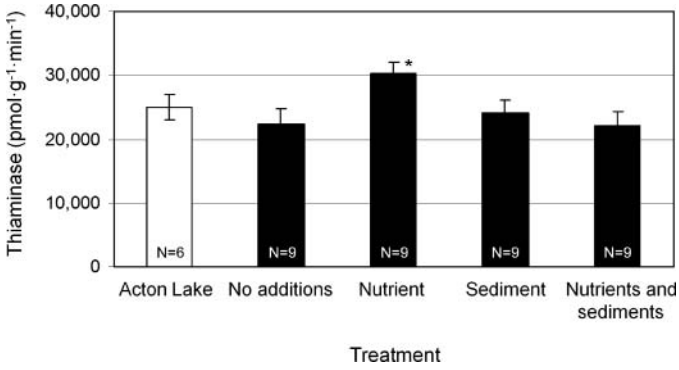

FIGURE 2. Least-squares means ± SE (accounting for the effect of batch) for thiaminase activity in Gizzard Shad (pmol thiamine·g^{-1}·min^{-1}, denoted as pmol·g^{-1}·min^{-1}) from the source population (open bar, Acton Lake) and the various treatments for the Gizzard Shad pond experiment (black bars). The number of fish tested (*N*) to establish each mean is shown at the base of the individual bars. The asterisk (*) denotes mean thiaminase activity that was significantly higher relative to other experimental ponds. Note the difference in scale of the *y*-axis relative to Figure 1.

temperatures in the treatment ponds (temperature was 1.2 ± 0.6°C, mean ± SD; below the lower lethal limit for Alewives observed in tank experiments by Colby 1973) were significantly colder (ANOVA: $F_3 = 442.4$, $P < 0.01$) than temperatures in the control ponds (temperature was 3.6 ± 0.9°C; temperatures where Alewives are routinely sampled in the Great Lakes). Despite significant differences in blood cell composition (approximately 22,000 lymphocytes/μL in fish from cold ponds versus 36,000 lymphocytes/μL in fish from control ponds) indicating there was stress associated with the experimental treatments (see Lepak and Kraft 2008), the mixed-model analysis that compared control and treatment groups showed no relationship between thiaminase activity and the prolonged exposure to cold temperatures (mixed-model: $F_{20, 20} = 0.37$, $P = 0.55$; see Figure 1). Mean water content of Alewives after the experiment was within 2% of the water content (70%) of the source population of Alewives from Waneta Lake (see Lepak and Kraft 2008), which was within the range of other Alewives previously measured in freshwater systems (Hartman and Brandt 1995), and did not differ significantly by treatment (Lepak and Kraft 2008). Similarly, mean thiaminase activity in the fish (approximately 5,200 pmol thiamine·g^{-1}·min^{-1}; Figure 1) after the experiment was within the range of thiaminase activities observed for wild-caught Alewife populations in freshwater systems (range, 1,700–7,000 pmol thiamine·g^{-1}·min^{-1}: Tillitt et al. 2005).

Gizzard Shad Pond Experiment

Gizzard Shad from the second batch of samples sent for thiaminase analysis lost a consistent amount of thiaminase while in storage (ANCOVA: $F_1 = 28.42$, $P < 0.01$). A multiple contrast comparison showed that least-squares means ± SE (accounting for the effect of batch) for thiaminase activities in Gizzard Shad were significantly higher in fish from ponds that were treated with nutrients (approximately 30,000 ± 1,800 pmol thiamine·g^{-1}·min^{-1}) relative to ponds with no nutrient or sediment additions (22,000 ± 2,500 pmol thiamine·g^{-1}·min^{-1}) and those that had sediments (24,000 ± 1,900 pmol thiamine·g^{-1}·min^{-1}) or nutrients plus sediments (22,000 ± 2,100 pmol thiamine·g^{-1}·min^{-1}) added (ANCOVA: $F_3 = 4.31$, $P = 0.01$; Figure 2).

Chlorophyll (μg/L), primary productivity (mg C·m^{-2}·d^{-1}), total phosphorus (μg P/L), and suspended solids (mg/L) were significantly lower in ponds with no nutrient or sediment additions relative to all other ponds (Pilati et al. 2009). Adult Gizzard Shad condition (Fulton's *K*) was significantly influenced by treatment (ANOVA: $F_3 = 87.60$, $P < 0.01$). Specifically, Fulton's *K* was lowest in ponds with no nutrient or sediment additions and significantly higher in the +S and +N+S treatments; *K* in the +N treatment was intermediate and not significantly different from the other treatments (Table 1). Thus, we observed the lowest *K* in control fish and the highest *K* in fish from the +N+S treatment, while fish with an intermediate *K* (+N treatment) had the highest thiaminase activities. Because thiaminase activity and condition factor were influenced by treatment, we conducted a comparison to evaluate whether thiaminase activity was related to condition in Gizzard Shad.

TABLE 1. Gizzard Shad Fulton's *K* (mean ± SD) by treatment.

Treatment	Mean ± SD Fulton's *K*
No additions	0.703 ± 0.071
Nutrient	0.795 ± 0.085
Sediment	0.807 ± 0.073
Nutrients and sediments	0.838 ± 0.069

An ANCOVA ($F_1 = 3.17$, $P = 0.09$) showed that the relationship was not significant at the level of $\alpha = 0.05$. These results were confirmed using a Monte Carlo randomization simulation showing that the relationship was significant at the level of $\alpha = 0.10$ (an F-value of 3.04 was required to confirm this) but not at $\alpha = 0.05$.

Importantly, excretion of P and N in Gizzard Shad was significantly higher (approximately 1.5–3 times higher) in fish from the ponds where nutrients were added relative to all other ponds (Pilati et al. 2009). Further, analyses of bulk sediment composition indicated that P, N, and organic C were the highest in ponds where nutrients were added relative to all other ponds (Pilati et al. 2009). The combination of these two observations indicates that the food available to Gizzard Shad in the ponds where nutrients were added was of higher quality relative to food in the other ponds. The thiaminase activities in fish from these ponds (those where nutrients alone were added) were significantly higher (30,000 $\pm$ 1,800 pmol thiamine$\cdot g^{-1}\cdot min^{-1}$, mean $\pm$ SE) than those from the other ponds (Figure 2).

DISCUSSION

The results observed in this study did not support the hypothesis that prolonged cold temperatures and low food availability produced an increase in clupeid thiaminase activity. Following the experiments, the thiaminase activity found in Alewives and Gizzard Shad held in pond systems was within the range of the original source populations. The only significant treatment effect on clupeid thiaminase was an observed increase in mean thiaminase activity in Gizzard Shad from the ponds where nutrients alone were added.

Alewives and Gizzard Shad held under conditions that were hypothesized to increase thiaminase activities (prolonged cold temperatures and low food availability) responded to experimental treatments in other ways. Alewives held in prolonged cold temperatures had significantly lower counts of circulating lymphocytes relative to Alewives held in warmer water temperatures (Lepak and Kraft 2008). Gizzard Shad that were held in ponds where nutrient and sediment additions were absent had significantly lower Fulton's K values and weighed less than Gizzard Shad held in ponds where nutrients, sediments, or both were added. These results were expected; however, no evidence was found that linked these responses to thiaminase activity in clupeids. Arguably this is evidence, in the case of the Alewife, that there is a disconnect between the immune response and thiaminolytic bacteria, or between thiaminolytic bacteria and thiaminase activity in Alewives.

Increases in mean thiaminase activities have been observed in Alewives held in captivity (replicated tanks) and provided a high quality diet in the form of commercial pellet food that was heat treated, thereby denaturing any thiaminase found in the feed (e.g., Lepak et al. 2008). Alewives maintained under laboratory conditions that were analyzed by Lepak et al. (2008) had thiaminase activities on the order of two- to three-fold higher than thiaminase activities in wild-caught Alewives (ranging from 1,700 to 7,000 pmol thiamine$\cdot g^{-1}\cdot min^{-1}$ in the wild: Tillitt et al. 2005; Lepak et al. 2008), but while in captivity these Alewives were not exposed to any known external sources of thiaminase (e.g., cyanobacteria, zooplankton, or other dietary sources). The clupeids in the current study were held in ponds where natural food sources were available instead of being provided heat-treated commercial pellet food. Again, the results of the pond study reported here support the laboratory experiment findings of Lepak et al. (2008) in that the mean thiaminase activities of Gizzard Shad with access to relatively high quality forage were elevated relative to those without access to high quality forage.

Gizzard Shad from the +N ponds were provided with high quality forage, which resulted in elevated P and N excretion rates and bulk sediment composition relative to the other ponds (Pilati et al. 2009). These shad had a mean thiaminase activity that was significantly higher than that for fish from the other ponds, supporting the notion that a high quality (high nutrient) diet was consumed by shad in ponds where only nutrients were added (Pilati et al. 2009). Gizzard Shad held in ponds where sediments were added were probably relying, at least to some extent, on these lower quality inputs for energy, experiencing a "dilution" effect on food quality (Heinrichs 1982; Mundahl and Wissing 1987; Higgins et al. 2006). Recent data from several Ohio reservoirs suggests that Gizzard Shad rely on energy derived from both phytodetritus and terrestrial detritus, but their biomass is higher in reservoirs where phytodetritus is the dominant energy source (Babler et al. 2011). Together these results suggest that food quality was highest in the +N ponds, where Gizzard Shad thiaminase activity was also highest.

The one consistent finding across the set of experiments described in this study and from similar data from tank experiments collected by Lepak et al. (2008) is that clupeids that had high quality diets available to them had higher thiaminase activities relative to those that did not. The observed mean water content, Fulton's K, and thiaminase activity of pond-reared Alewives and Gizzard Shad in this study were within the range of wild-caught fish (Hartman and Brandt 1995; Tillitt et al. 2005; Pilati et al. 2009). However, the mean water content values of Alewives held in the laboratory by Lepak et al. (2008) were lower than any other previously reported values (Hartman and Brandt 1995), yet thiaminase activities were consistently higher than for any Alewives previously evaluated from natural freshwater systems (Tillitt et al. 2005).

Although the isolation of thiaminase-positive bacteria from Alewife viscera has been described as the potential source of clupeid thiaminase (Honeyfield et al. 2002), the sources of observed thiaminase activity in Alewives remain uncertain (Richter et al. 2012). Bacterial communities and their gene expression within fish viscera are altered by different feeding regimes and fish condition (Šyvokienė and Mickėnienė 1999). The results of our experiments suggest that clupeids feeding on high quality food sources sustain high levels of thiaminase activity that could be

fostered by internal conditions in clupeids that affect bacterially produced thiaminase. Our results do not discount the possibility that clupeid physiology may be influenced by feeding conditions, and if clupeids produce thiaminase, feeding conditions could influence thiaminase activity. However, we speculate that bacteria in clupeids react similarly to bacteria in other organisms. For example, thiaminolytic bacteria can proliferate in the gastrointestinal tracts of ruminants under certain feeding regimes (increased carbohydrate consumption), ultimately resulting in thiamine deficiency (Brent 1976). We note other observations in the animal husbandry literature regarding the increase in thiaminase production in the presence of high quality feed when a sudden change in rumen pH occurred (Brent and Bartley 1984; Zinn et al. 1987). The gastrointestinal tract of fish provides a similar environment with abundant thiamine during periods of active feeding on high quality forage, and the transition between the stomach and intestine provides a location where pH can rapidly change from <6 to >7, mimicking conditions found in ruminants suffering from thiamine deficiency.

Although thiaminases have been generally considered in the fisheries literature for their role in degrading available thiamine, one form of thiaminase (thiaminase II, now referred to as transcriptional enhancer A or "TenA") can function in thiamine synthesis by salvaging pyrimidine from ring-opened thiamine (Toms et al. 2005; Jenkins et al. 2007). Jenkins et al. (2007) and Bettendorff (2007) suggest that the primary function of thiaminase II is associated with thiamine biosynthesis by bacteria, rather than destruction. In light of these findings, Soriano et al. (2008) proposed that the evolution of thiamine-degrading activity by thiaminase I might be linked to a salvage pathway that recycles degraded forms of thiamine.

Thiamine degradation occurs under environmental conditions in which pH > 7 (Maier and Metzler 1957); therefore, fish gastrointestinal tracts with pH > 7 could be conducive to thiaminase-producing bacteria capable of utilizing degraded forms of thiamine. In our study, Gizzard Shad from environments where high quality forage was available were observed to have the highest levels of thiaminase activity. Similarly, we obtained results in a laboratory study in which we found that Alewives held in captivity and fed high quality diets in the form of commercial pellet feed had greater thiaminase activities than those previously reported (Lepak et al. 2008). We suggest that consumption of high quality food sources could have led to the production of thiamine degradation products that are salvaged for thiamine synthesis by bacteria, fostering conditions that trigger the production of bacterial thiaminase. Thus, if thiaminolytic bacteria are the primary source of thiaminase in clupeids (not clupeids themselves), it is possible that clupeid feeding conditions could result in altered expression of thiaminase or the proliferation of thiaminolytic bacterial populations, or both.

Progress in understanding TDC in valuable fisheries will require a better understanding of several phenomena stemming from the observation that thiaminase has been primarily found in the visceral tissues of fish and only small amounts of thiaminase have been detected in muscle tissue (Fujita 1954). First, we need to determine the primary sources of fish thiaminase activity. Then, we need to determine the environmental factors that regulate and affect production of thiaminase by bacteria or fish themselves, or both. Finally, we need a model fish system in which thiaminase activity can be manipulated in order to document the conditions that affect thiaminase activity in these organisms. If thiaminase is produced primarily by bacteria within fish, we will need a broader understanding of the varieties and abundance of thiaminase I-producing bacteria present in specific environmental conditions, (e.g., within fish gastrointestinal tracts). Thus, the characterization of internal clupeid microbial communities and their expression of thiaminase (or lack thereof) will provide important insights into thiaminase research, help determine the ultimate sources of thiaminase in fish, and help focus future research.

Understanding the dynamics of thiaminase expression in clupeids will aid ongoing efforts to reestablish sustainable, naturally reproducing salmonine communities in the Great Lakes and maintain naturally reproducing salmonine communities around the world. Given the dependency of salmonines on clupeids as forage, the ongoing spread of clupeids across the United States and Canada, and the threat of thiamine deficiency associated with clupeids to salmonine natural reproduction, it will be important for scientists, managers, and stakeholders to recognize and acknowledge the importance of these prey fish. For example, further introductions of Alewives carry serious, yet unpredictable, implications related to salmonine communities because of variation in Alewife population size and thiaminase content. Continuing efforts to evaluate clupeid thiaminase variability and the use of whole-system manipulations and other innovative techniques will provide applicable results that could lead to the remediation of the negative impacts that clupeids have on salmonines and possibly other predators.

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