

Thiamine and Thiaminase Status in Forage Fish of Salmonines from Lake Michigan

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Abstract.—Dietary sources of thiamine (vitamin B₁) and thiamine-degrading enzymes (thiaminases) are thought to be primary factors in the development of thiamine deficiency among Great Lakes salmonines. We surveyed major forage fish species in Lake Michigan for their content of thiamine, thiamine vitamers, and thiaminase activity. Concentrations of total thiamine were similar ($P \leq 0.05$) among most forage fishes (alewife *Alosa pseudoharengus*, bloater *Coregonus hoyi*, spottail shiner *Notropis hudsonius*, deepwater sculpin *Myoxocephalus thompsonii*, yellow perch *Perca flavescens*, ninespine stickleback *Pungitius pungitius*, and round goby *Neogobius melanostomus*) and slightly lower in rainbow smelt *Osmerus mordax*. Concentrations of total thiamine were all above the dietary requirements of coldwater fishes, suggesting the thiamine content of forage fish is not the critical factor in the development of thiamine deficiency in Lake Michigan salmonines. Thiamine pyrophosphate was the predominant form of thiamine in most species of forage fish, followed by free thiamine and thiamine monophosphate. Total thiamine was slightly greater in summer collections of alewife and rainbow smelt than in spring and fall collections, but the same was not true for bloater. Thiaminase activity varied among species and was greatest in gizzard shad *Dorosoma cepedianum*, spottail shiner, alewife, and rainbow smelt. Thiaminase activity in alewife varied among collection locations, season (greatest in spring), and size of the fish. Size and condition factors were positively correlated with both total thiamine and thiaminase activity in alewife. Thus, thiamine and thiaminase activity in forage fishes collected in Lake Michigan varied among species, seasons, year caught, and size (or condition). Therefore, multiple factors must be considered in the development of predictive models for the onset of thiamine deficiency in Great Lakes salmonines. Most importantly, thiaminase activity was great in alewives and rainbow smelt, suggesting that these prey fish are key causative factors of the thiamine deficiency in Great Lakes salmonines.

Thiamine (vitamin B₁) deficiency in Great Lakes salmonines is thought to result from a diet that

contains a high proportion of certain exotic species of forage fish, such as alewife *Alosa pseudoharengus* and, possibly, rainbow smelt *Osmerus mordax* (Fitzsimons 1995; Fitzsimons et al. 1999). These forage species contain thiaminase from thiaminase-producing bacteria (Honeyfield et al. 2002) and possibly other sources, which can de-

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grade thiamine in predatory species such as salmonines. The consequences of thiamine deficiency in Great Lakes salmonines have included avitaminosis in adults (Brown et al. 2005, this issue) and early mortality syndrome (EMS) in developing fry (Marcquenski and Brown 1997). Thiamine deficiency has been observed in various species of salmonines in the Great Lakes. Notably, coho salmon *Oncorhynchus kisutch* and lake trout *Salvelinus namaycush* from Lake Michigan have been affected to the point where their populations may be threatened (Fitzsimons et al. 1999). Moreover, evidence suggests that thiamine deficiency is a contributing factor and, possibly, a major factor in the lack of recruitment observed in Lake Michigan lake trout. Thiamine deficiency has also been observed in Atlantic salmon *Salmo salar* and brown trout *Salmo trutta* from the Baltic Sea (Amcoff et al. 1999); the presence of thiaminase activity in the main forage fish, the Baltic herring *Clupea harengus membras*, is suspected to be the causative agent (Wistbacka et al. 2002).

The patterns and intensities of thiamine deficiency and subsequent EMS have varied in Great Lakes salmonines. The cause or causes of these variations are unknown. The degree of thiamine deficiency and EMS in coho salmon populations has generally increased over the past two decades, but year-to-year variations (up to 90%) have occurred (Wolgamood et al. 2005, this issue). The amount of EMS observed in coho salmon taken in the fall of 1999 at the Platte River was approximately 5%, while the following year the Michigan Department of Natural Resources (MDNR) observed a 90% occurrence of EMS in coho salmon taken from the same river (Wolgamood et al. 2005). The variation of thiaminase activity in forage fishes that the salmonines prey upon may be the proximate cause for the observed variation in thiamine deficiency. Therefore, to begin to understand the degree and extent of variation in thiamine and thiaminase activity of Great Lakes prey species, measurements were made on Lake Michigan prey species. We collected a variety of prey species of different sizes from various locations during three different seasons. Our intent was to identify differences in thiamine content and thiaminase activity that may be related to these factors.

Methods

Prey Species

The focal prey species examined were rainbow smelt, bloater *Coregonus hoyi*, and alewife. In ad-

dition to these species, we also collected limited numbers of spottail shiner *Notropis hudsonius*, deepwater sculpin *Myoxocephalus thompsonii*, gizzard shad *Dorosoma cepedianum*, ninespine stickleback *Pungitius pungitius*, yellow perch *Perca flavescens*, and round goby *Neogobius melanostomus* for examination and analysis.

Experimental Design

Collections were designed to evaluate variations in thiamine content and thiaminase activity of prey species based on differences in species, size, location, season, and year. Samples of forage fishes were collected from Lake Michigan near Saugatuck, South Haven, St. Joseph, Muskegon, Grand Traverse Bay, Manistique, and Two Rivers, Michigan, and Sturgeon Bay, and Port Washington, Wisconsin. Sampling was conducted during the spring (April–May), summer (June–August), and fall (September–October) of 1998, 1999, and 2000. Size-classes were preselected for alewife (<60 mm, 60–120 mm, and >120 mm), rainbow smelt (<100 mm and >100 mm), and bloater (<120 mm and >120 mm), while all other species were opportunely sampled. Measurements made after collection consisted of fish weight (W ; g), total length (L ; mm or cm), and the subsequent calculation of Fulton's condition factor ($K = 100 W/L^3$). Samples were collected with 12-m otter trawls for periods of not more than 30 min and quick frozen on dry ice (Wright et al. 2005, this issue). Fish were shipped on dry ice and then stored at -80°C until processed for analysis of thiamine vitamers and thiaminase.

Tissue Preparation

All materials and equipment used for pulverizing and grinding were stored overnight at -20°C . Whole fish were pulverized at -20°C and ground at 4°C with dry ice. Frozen fish were broken into manageable pieces, chopped with a blade into smaller pieces (1–2 cm), and then ground to a fine powder with a mortar and pestle, all in the presence of dry ice (for details, see Zajicek et al. 2005, this issue). Once the entire sample was ground, it was stored in a ziplock bag at -80°C until analysis. Small fish (<5 g) were simply ground with dry ice with a mortar and pestle.

Thiamine Vitamers

Concentrations of thiamine vitamers were determined with a specific high-performance liquid chromatography procedure that separated phosphorylated and nonphosphorylated thiamine forms

TABLE 1.—Total thiamine and thiaminase activity in forage fish collected from Lake Michigan. Values are lakewide means of all samples collected over the study period (1998–2000); SDs are given in parentheses. Values with different letters within a column are significantly different ($P = 0.05$); NA = not analyzed.

Species	Total thiamine		Thiaminase	
	<i>N</i>	Content (nmol/g)	<i>N</i>	Activity (pmol·g ⁻¹ ·min ⁻¹)
Alewife	403	15.5 (7.9) zy	385	4,280 (2,330) y
Rainbow smelt	125	8.8 (6.7) y	120	2,640 (2,120) y
Bloater	71	14.2 (8.0) z	57	35 (38) wvu
Gizzard shad		NA	4	31,800 (4,260) z
Spottail shiner	16	16.3 (4.7) z	16	32,700 (17,600) z
Deepwater sculpin	54	16.5 (8.4) zy	54	172 (346) wv
Yellow perch	6	26.4 (8.4) z	6	12 (6) vu
Ninespine stickleback	3	25.7 (6.9) z	3	85 (63) x
Round goby	6	14.6 (3.7) zy	6	18 (8) u

with fluorescence detection (Brown et al. 1998). Free thiamine (FT; nonphosphorylated form), thiamine monophosphate (TMP), thiamine pyrophosphate (TPP), and total thiamine vitamers (sum of the individual forms) were reported in units of nanomoles per gram.

Thiaminase I

The catalytic activity of thiaminase I was measured by liberation of 2-¹⁴C-thiazole radioactivity from [thiazole-2-¹⁴C] thiamine at pH 6.5 at 37°C. The optimized procedure is fully described in Zajicek et al. (2005). Briefly, a dilution series of tissue homogenate was incubated with [thiazole-2-¹⁴C] thiamine and the base cofactor nicotinic acid for 10 min before halting the reaction and extracting the 2-¹⁴C-thiazole radioactivity with ethyl acetate. The extract was counted by liquid scintillation counting to determine the breakdown of thiamine. Thiaminase values were reported as picomoles of thiamine degraded per gram of tissue per minute of incubation (pmol · g⁻¹ · min⁻¹).

Statistics

Data analysis was conducted with SAS statistical software (SAS 1990). Statistical significance was set at the 0.05 level for all analyses. Data analysis was performed on transformed data (log₁₀) to ensure the data were normally distributed through a Kolmogorov–Smirnov test. Comparisons of mean values were made with Duncan's multiple-range test or least-squares method. Relationships between variables were evaluated by Pearson's product-moment correlation coefficient analysis. Evaluation of more complex relationships among variables (collection site, season, species, and year) was conducted with SAS (1990) general linear model (GLM) procedures in a nested-design analysis of variance (ANOVA).

Results

Species

The content of total thiamine vitamers was similar in all of the species of forage fish examined except rainbow smelt (Table 1). Rainbow smelt had a mean total thiamine content of 8.8 nmol/g in whole fish, which was far lower than that found in bloater, spottail shiner, yellow perch, and ninespine stickleback, but not different from alewife, deepwater sculpin, or round goby (Duncan's multiple-range test). The range of mean concentrations of thiamine vitamers for forage fish species other than rainbow smelt was relatively small, 14.2–26.4 nmol/g (Table 1).

The individual vitamers of thiamine in forage species had differential distribution patterns (Figure 1). Thiamine pyrophosphate was the major form of thiamine (47–74% total thiamine) in all species except the round goby in which TPP was 40% of the total thiamine content. The mean concentration of TPP in whole fish from Lake Michigan ranged from 5.3 nmol/g in round goby to 16.5 nmol/g in yellow perch. Free thiamine was the second most abundant form of thiamine vitamers (16–37% total thiamine), again with the exception of the round goby in which FT was the most abundant form of thiamine (56% total thiamine). Mean concentrations of FT in forage species had a range of 1.2 nmol/g in rainbow smelt to 8.7 nmol/g in round goby. The least abundant form of thiamine (4–19% total thiamine) was TMP in all species except deepwater sculpin, in which TMP was not significantly different from FT.) Given the differences in general proportions of the thiamine vitamers, there were also significant differences in the actual concentrations of the vitamers among species (Figure 1).

Thiaminase activity varied significantly among

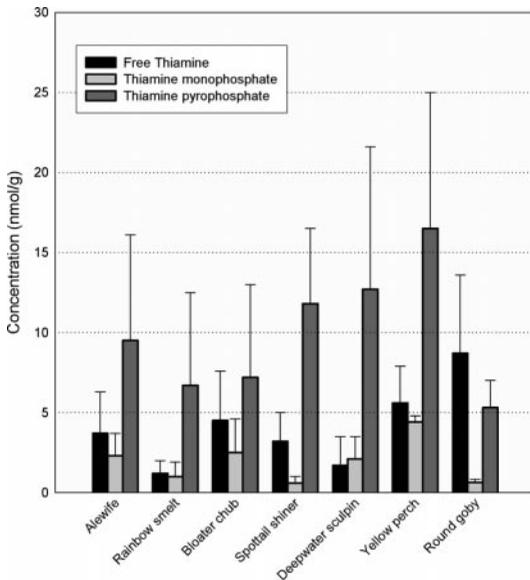


FIGURE 1.—Thiamine vitamers concentrations in forage fish collected from Lake Michigan. Values are lake-wide means of all samples collected over the study period (1998–2000). See Table 1 for sample sizes for each species.

the forage species collected in Lake Michigan (Table 1). The lowest thiaminase activities ($12\text{--}35 \text{ pmol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$) were measured in yellow perch, round goby, and bloater; there were no significant differences observed among these three species (Duncan's multiple-range test). The mean amount of thiaminase activity in these three species was near the limit of detection for the thiaminase assay (approximately $5 \text{ pmol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$). Slightly greater than these three species was the mean thiaminase activity of ninespine stickleback at $85 \text{ pmol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$ and deepwater sculpin at $172 \text{ pmol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$ (Table 1). The amount of thiaminase activity observed in the deepwater sculpin and ninespine stickleback is still considered low, even though the concentrations were detectable. Thiaminase activity in alewife (mean = $4,280 \text{ pmol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$) and rainbow smelt (mean = $2,640 \text{ pmol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$) were significantly greater than the aforementioned species, but not significantly different from one another (Table 1). Thiaminase activity in alewife was consistently greater than that observed in rainbow smelt at a given location or collection season (Table 2), but the amount of variation in thiaminase measurements and among individuals within a location made these differences statistically not significant. The greatest amount of thiaminase activity detected in

Lake Michigan forage fish species was measured in gizzard shad ($31,800 \text{ pmol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$) and spottail shiner ($32,700 \text{ pmol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$; Table 1). These values were not different from one another but were significantly greater than the thiaminase activity of all of the other species examined.

Location

The focal species of this study, alewife, rainbow smelt, and bloater, were collected from 11 locations over the course of these studies (Table 2). These collections were, in turn, focused on the southeastern quadrant (Saugatuck, Grand Haven, Muskegon, South Haven, and St. Joseph, Michigan) and northwestern quadrant (Sturgeon Bay and West Island, Wisconsin, and Manistique and Two Rivers, Michigan) of Lake Michigan to determine whether a simple regional difference in thiamine or thiaminase activity in forage fish could be observed. Simple regional-based differences in either thiamine content or thiaminase activity in Lake Michigan forage fish species were not evident from these investigations (Duncan's multiple-range test). The mean concentrations of total thiamine, thiamine vitamers, and thiaminase activity in alewife from collection locations within these regional quadrants were variable (Table 2; GLM). The proportions of the thiamine vitamers (FT, TMP, and TTP) relative to concentrations of total thiamine varied among alewives as they varied among rainbow smelt caught at different locations (GLM). The thiaminase activity measured in rainbow smelt collected from locations across Lake Michigan did not vary (GLM) and had a mean activity of $2,640 \text{ pmol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$. Total thiamine and thiamine vitamers content of bloaters varied significantly among collection locations (Table 2; GLM). The thiaminase activity of bloaters was consistently small at all of the locations in Lake Michigan (Table 2).

Season

Season of the year (spring, summer, or fall) in which the forage fish were collected had a significant effect on both total thiamine vitamers (data not shown) and thiaminase activity in alewife (Figure 2). Concentrations of total thiamine tended to be lowest in spring-caught alewife, slightly elevated in the summer, and slightly reduced in the fall. The concentrations and relative proportions of the thiamine vitamers measured in alewife also varied among seasons (GLM); the exception was TMP, which had mean concentrations of 2.1–2.3

nmol TMP/g across all three seasons. Mean concentrations and relative proportions of TPP in alewives collected in Lake Michigan across locations were lowest in the spring (5.7 nmol TPP/g; 45%) and similar in the summer (10.3 nmol/g; 58%) and fall (9.0 nmol TPP/g; 59%). Mean concentrations of FT in alewife across locations in Lake Michigan were lowest in the fall (2.9 nmol FT/g; GLM) and similar in the spring (4.4 nmol FT/g) and summer (3.8 nmol FT/g). The mean proportions of FT were greatest in spring-caught alewife (36%) and similar in summer-caught (27%) and fall-caught (24%) alewives. The thiaminase activity in alewife was greatest in the spring, lowest in the summer, and intermediate in the fall (Figure 2).

The total thiamine content of rainbow smelt followed a similar seasonal trend to that of alewife: lowest in spring, greatest in the summer, and lower in the fall. The thiamine vitamers measured in rainbow smelt also varied among season of collection (GLM) in a similar fashion: smallest in the spring, greatest in the summer, and intermediate in the fall. The thiaminase activity in rainbow smelt did not have significant seasonal differences when considered across all locations sampled (mean range, 2,560–2,790 pmol · g⁻¹ · min⁻¹; Figure 2).

Total thiamine vitamers measured in bloater did not vary as a function of season. Thiaminase activity of bloaters collected during the summer was statistically greater than the other seasons, but the thiaminase activities of bloater were all extremely low and the differences among seasons were not thought to be biologically relevant (Table 2).

Year

The year-to-year variation in thiamine vitamers and thiaminase activity was evaluated in alewife, rainbow smelt, and bloater. Year-to-year variations were observed in concentrations of thiamine vitamers, the relative proportions of the vitamers, and in thiaminase activity measured in alewives collected from Lake Michigan in 1998, 1999, and 2000 (GLM). The lakewide mean value for concentrations of total thiamine in alewife was 17.7 nmol/g in 1998, down to 11.5 nmol/g in 1999, and up to 18.2 nmol/g in 2000 (data not shown). Concentrations of TPP and TMP in alewife followed this same pattern (lowest lakewide mean value in 1999), while the concentration of FT varied but not in this same pattern. The mean thiaminase activity in alewives collected at all locations of Lake Michigan was 5,090 pmol · g⁻¹ · min⁻¹ in 1998, 30% smaller in 1999 (3,560 pmol · g⁻¹ · min⁻¹),

and then 4,000 pmol · g⁻¹ · min⁻¹ in alewives collected in the year 2000 (Figure 2).

The yearly mean value of total thiamine in rainbow smelt varied among the three years of collection (GLM), as did the individual vitamers. Concentrations of total thiamine, TMP, and TPP in rainbow smelt from Lake Michigan were similar in 1998 and 1999, but dramatically increased in 2000. The mean value of total thiamine in rainbow smelt was 4.1 nmol/g in 1998 and 4.0 nmol/g in 1999 and then increased to 15.1 nmol/g in rainbow smelt collected in the year 2000. Mean TPP in rainbow smelt appeared to be the main reason for this increase observed in total thiamine, as concentrations of TPP increased from 2.5 to 2.7 nmol/g in 1998–1999 and reached 12.1 nmol/g in 2000. The activity of thiaminase contained in the whole carcasses of rainbow smelt did not vary over these same three years of collection (Figure 2).

A variation was also observed in the mean thiamine content of bloaters caught in different years from Lake Michigan (GLM). Total thiamine concentration was 15.5 nmol/g in bloaters caught in 1998 and then dropped to a mean value of 5.5 nmol/g in 1999. These differences were mainly caused by differences in TPP form of the vitamin, as FT did not vary across years. Thiaminase activity in bloater was not measured after 1998 because of the extremely low activity measured in this species.

Size

The effect of forage fish size on total thiamine content and thiaminase activity was investigated by the collection of fish from at least three distinct size-classes within the three focal species. It was often not possible to obtain fish from each of the size-classes during a given collection or at certain times of the year. The weight and total length of forage fishes were subject to correlation analysis against total thiamine content or thiaminase activity to determine any simple, linear relationships. Fish weight was used as the descriptive metric for correlation analyses in all cases below for ease of presentation. All correlations that were significant when measured against weight were also significant when measured against fish length. The differences in species and seasonal values for both thiamine and thiaminase (mentioned above) made it requisite to conduct the comparisons by both season and species.

There was a significant correlation between fish weight and total thiamine in alewives caught in the summer ($r = 0.31$, $P < 0.0001$), while no such

TABLE 2.—Total thiamine content and thiaminase activity measured in alewife, rainbow smelt, and bloater collected from locations in Lake Michigan during 1998–2000. Standard deviation (in parentheses) follows the mean value. Values without parentheses are single values ($N = 1$). Blanks represent no sample taken; NA = not analyzed.

Region	Location	Season and year	Alewife	
			Thiamine (nmol/g)	Thiaminase (pmol·g ⁻¹ ·min ⁻¹)
SE	Saugatuck, Michigan	Spring 1998	9.7 (3.4)	5,760 (2,170)
		Spring 1999	14.2 (3.6)	7,880 (2,040)
		Fall 1999	3.3 (1.0)	4,310 (1,370)
		Spring 2000	11.1 (2.0)	5,170 (2,170)
SE	Grand Haven, Michigan	Summer 2000		
SE	Muskegon, Michigan	Summer 2000	21.4 (6.6)	3,370 (1,500)
SE	South Haven, Michigan	Summer 1998	11.7 (5.1)	4,420 (1,850)
		Fall 1998	13.9 (2.6)	3,340 (1,780)
		Spring 1999	9.4	1,760
		Summer 1999	16.0 (8.2)	3,010 (1,630)
		Summer 2000	14.1 (6.3)	2,580 (1,270)
SE	St. Joseph, Michigan	Summer 1999	21.1 (11.2)	3,910 (1,800)
SW	Port Washington, Wisconsin	Fall 1998	15.4 (7.5)	5,850 (2,200)
NW	Sturgeon Bay, Wisconsin	Spring 1998	13.7 (4.6)	6,180 (2,370)
		Summer 1998	17.1 (5.1)	4,570 (1,850)
		Summer 1999	14.1 (5.0)	2,940 (1,490)
		Fall 1999	18.5 (6.4)	4,440 (2,390)
		Spring 2000	15.7 (3.9)	8,640 (3,150)
		Summer 2000	24.0 (6.5)	4,540 (2,130)
		Fall 2000	18.1 (6.8)	3,740 (2,900)
NW	Manistique, Michigan	Summer 1988	24.8 (5.4)	5,750 (2,500)
		Summer 1999	6.7 (2.0)	2,600 (1,850)
NW	Two Rivers, Michigan	Fall 1998	18.5 (1.7)	7,780 (2,880)
NW	West Island, Wisconsin	Summer 1999	7.1 (2.1)	2,230 (1,140)
NE	Grand Traverse Bay, Michigan	Summer 1998	21.6 (6.6)	4,890 (1,800)

correlation was observed in alewives caught in the spring and fall. Alewife weight was also positively correlated with thiaminase activity in the summer ($r = 0.45$; $P < 0.0001$) and in the fall ($r = 0.25$; $P = 0.03$). However, thiaminase activity was not correlated with weight in spring-caught alewives. The condition of alewives (Fulton's condition factor) was also significantly correlated with the activity of thiaminase measured in alewives from Lake Michigan; however, K only explained a small portion of the variance ($r = 0.20$; $P \leq 0.001$) over the entire range of the alewife data set.

Thiamine content of rainbow smelt had no correlation with fish size (length or weight) in the summer or fall and was negatively correlated with fish size in spring-caught fish ($r = -0.53$; $P = 0.008$). Thiaminase activity in rainbow smelt was not correlated with fish size in any time of the year. No significant correlations were observed between fish size and thiamine content or thiaminase activity in bloaters caught during any time of the year.

Thiamine–Thiaminase

The relationship between thiamine content and thiaminase activity was explored in the three focal

species, alewife, rainbow smelt, and bloater, during the three observational seasons. Alewife had a positive correlation between thiamine content and thiaminase activity in both spring-caught ($r = 0.33$; $P = 0.014$) and summer-caught ($r = 0.26$; $P < 0.0001$) fish. This correlation between thiamine content and thiaminase activity was not observed during the fall season. No significant correlations were observed between thiamine and thiaminase in rainbow smelt. Bloater had a significant correlation between thiamine content and thiaminase activity in spring-caught fish ($r = 0.57$; $P = 0.03$), but no significant relationship was observed in the summer or fall for this species.

Discussion

Mean concentrations of total thiamine in forage fishes varied little among the eight forage fish species examined in these studies. The mean total thiamine content of rainbow smelt was the lowest of the forage fish species and was significantly smaller than many other species. Fitzsimons et al. (1998) found muscle concentrations of thiamine to be the lowest among alewife, bloater, cisco *Coregonus artedii*, and rainbow smelt collected from either Lake Michigan or Lake Ontario. These au-

TABLE 2.—Extended.

Location	Season and year	Rainbow smelt		Bloater			
		Thiamine (nmol/g)	Thiaminase (pmol·g ⁻¹ ·min ⁻¹)	Thiamine (nmol/g)	Thiaminase (pmol·g ⁻¹ ·min ⁻¹)		
Saugatuck, Michigan	Spring 1998	2.6 (1.6)	2,870 (892)	13.3 (3.1)	15 (7)		
	Spring 1999	1.0 (0.2)	1,670 (400)				
	Fall 1999	1.5 (0.6)	2,730 (4,140)				
	Spring 2000	9.8 (3.3)	2,020 (476)				
	Summer 2000	12.1	1,500				
Grand Haven, Michigan	Summer 2000	13.9 (4.5)	2,460 (1,890)	16.3 (6.8)	58 (48)		
Muskegon, Michigan	Summer 1998						
South Haven, Michigan	Fall 1998						
	Spring 1999						
	Summer 1999						
	Summer 2000	23.3 (1.7)	345 (106)				
St. Joseph, Michigan	Summer 1999			9.0 (2.5)	NA		
Port Washington, Wisconsin	Fall 1998	9.0 (1.8)	1,800 (879)				
Sturgeon Bay, Wisconsin	Spring 1998	3.4 (1.9)	2,950 (1,450)			11.7 (2.0)	10 (1)
	Summer 1998					10.5 (5.6)	23 (17)
	Summer 1999						
	Fall 1999	8.7 (2.1)	3,150 (2,940)				
	Spring 2000						
	Summer 2000	15.9 (3.8)	3,420 (1,910)				
	Fall 2000	19.2 (3.4)	2,640 (1,030)				
Manistique, Michigan	Summer 1988			26.9 (8.3)	16 (10)		
	Summer 1999	1.6 (0.9)	2,530 (1,150)	5.3 (1.1)	NA		
Two Rivers, Michigan	Fall 1998			26.0 (4.9)	37 (38)		
West Island, Wisconsin	Summer 1999	4.1 (1.7)	1,600 (1,380)	7	NA		
Grand Traverse Bay, Michigan	Summer 1998						

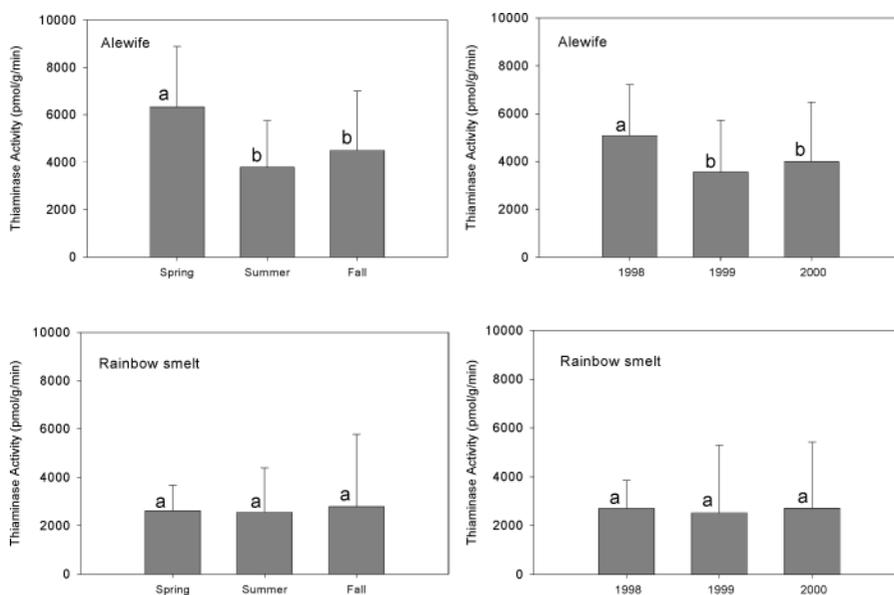


FIGURE 2.—Seasonal and yearly mean values of thiaminase activity in alewife and rainbow smelt collected from Lake Michigan in 1998–2000. Values are the means for a species collected from all locations across Lake Michigan. Values with different letters within a species are significantly different (general linear model: $P < 0.05$).

thors measured thiamine vitamers in muscle tissue from alewives (11.1 nmol/g), slimy sculpins (10.9 nmol/g), bloaters (5.8 nmol/g), and rainbow smelt (2.5 nmol/g) collected in the fall of 1995 from Lake Michigan. The values of total thiamine were within the same range that we observed, only all values from the earlier study were less than those presented here (Table 1). The differences in concentrations of total thiamine measured in the same species is probably a result of the differences in the tissues that were analyzed in the two research efforts (whole fish in this report versus muscle tissue in the Fitzsimons et al. 1998 study). Thiamine content in muscle is often less than in other tissues of fish (Halver 1989).

Thiamine pyrophosphate was the predominant form of thiamine measured in whole carcasses of forage fish species collected in Lake Michigan (TPP, 47–74%); the exception was round goby (TTP, 40%). There were location, seasonal, and yearly differences in the relative ratios of FT, TMP, and TPP, but these differences in relative proportions of the thiamine vitamers were generally small. The relative abundance of the thiamine vitamers from greatest to least was TPP–FT–TMP in all species, except round goby in which the relative abundance was FT–TPP–TMP and deep-water sculpin with a relative abundance of TPP–TMP–FT (Figure 1). The relative abundance of thiamine vitamers in muscle samples of alewives, rainbow smelt, and bloaters collected from Lake Michigan in 1995 was TPP–TMP–FT (Fitzsimons et al. 1998). The greatest variation in relative abundance of the thiamine vitamers among the target species was observed in bloater (Figure 1). Abundance of FT was greater than other thiamine vitamers in bloaters collected in Lake Michigan near Saugatuck (spring 1998), Sturgeon Bay (spring 1998), Manistique (summer 1999), and West Island (summer 1999; data not shown). It is interesting to note that even though the relative abundance of the thiamine vitamers varies, the concentrations of FT and TMP remained fairly constant within a species. The largest differences in the relative proportions of thiamine vitamers were generally seen in the TPP content. Thiamine pyrophosphate accounted for most of the year-to-year differences noted in alewife, rainbow smelt, and bloater (data not shown). These differences in TPP content may be significant with respect to the thiamine status of these species. Thiamine pyrophosphate is the active form of the metabolic cofactor (Halver 1989) and, as such, changes in con-

centrations of TPP will have more pronounced effects on thiamine-dependent enzymes.

The thiamine content of the target species varied among collection locations in Lake Michigan (Table 2), season of the year (Figure 2), and year of collection (Figure 2). These differences observed in thiamine content, however, were not always the same in direction or magnitude among alewife, rainbow smelt, and bloater. For example, mean concentrations of total thiamine in alewife and rainbow smelt were greatest in the summer, while no seasonal differences were observed in bloater (Table 2). This point is highlighted in instances where species were co-collected (Table 2). For example, there was a relative increase in total thiamine concentrations in alewives collected near Saugatuck in the spring of 1998 (9.7 nmol/g) compared with the next year at the same location (spring 1999, 14.2 nmol/g). At the same times and locations, concentrations of total thiamine in rainbow smelt went down (spring 1998, 2.6 nmol/g; spring 1999, 1.0 nmol/g; Table 2). Concentrations of total thiamine in alewives and rainbow smelt collected at Sturgeon Bay (summer 2000 and fall 2000) also went in opposite directions, only this time decreasing in alewife and increasing in rainbow smelt (Table 2). Changes in thiamine concentrations (both seasonally and across years during the same season) that go in the opposite directions in different species indicate that the factors that control thiamine content are different among these species. This is not unexpected in the sense that alewife and rainbow smelt in Lake Michigan are known to have differential feeding niches and temperature preferences (Foltz and Norden 1977; Janssen and Brandt 1980).

Regardless of the differences noted in the measured concentrations of thiamine in forage fish species collected from Lake Michigan, all of the species contained thiamine concentrations that were much greater than the presumed dietary requirements of 1.0 nmol/g for salmonine species and EMS (Brown et al. 2005) or that estimated by Halver (1989) for adult fish health (3.0 nmol/g). Thus, the total thiamine content of the forage fishes does not appear to be the critical or limiting factor for the observed thiamine deficiency that occurs in Lake Michigan salmonines.

Thiaminase Activities in Forage Species

Thiaminase in the diets of salmonines is thought to be a causal agent in the development and onset of thiamine deficiency in adults and EMS in offspring of thiamine-deficient broodstock (Fisher et

al. 1995; Fitzsimons 1995; Fitzsimons et al. 1998; Ji and Adelman 1998). Indeed, dietary thiaminase can cause thiamine deficiency in fishes in laboratory experiments (Wolf 1942; Harrington 1954; Ishihara et al. 1978; Ji et al. 1998). Salmonines are particularly sensitive to dietary thiaminase and subsequent thiamine deficiency. Recently, detailed studies have documented the quantitative relationship between thiaminase activity in the diets of lake trout broodstock and the development of thiamine deficiency, including EMS in fry (Honeyfield et al. 2005, this issue). Thus, a better understanding of the fluctuations in thiaminase activity in forage fishes of the Great Lakes and the factors that influence that activity will be vital to understand and predict the impact of thiaminase on populations of lake trout in Lake Michigan and the other Great Lakes. Our measurements of thiaminase activity in major forage species of salmonines were designed to provide insight into these fluctuations.

Thiaminase activity varied significantly among species of forage fish collected from Lake Michigan. The species that had the lowest thiaminase activities (deepwater sculpin, bloater, ninespine stickleback, and yellow perch) were all native species to Lake Michigan. Low (or no) thiaminase activity has been reported in whole-body analyses of yellow perch collected from Lake Michigan (Deutsch and Hasler 1943) and Nova Scotia (Neilands 1947). Viscera of yellow perch contained some thiamine-destroying ability that was presumed to result from dietary sources (Arsan 1970). Bloater contained no appreciable amounts of thiaminase activity in our studies (Table 1) or in previous reports (Grieg and Gnaedinger 1971). No studies were found that examined thiaminase activity in deepwater sculpin; however, fourhorn sculpin *Myoxocephalus quadricornis* collected from Lake Michigan in the late 1960s to early 1970s contained an unreported but "positive" amount of thiaminase activity (Grieg and Gnaedinger 1971). The species with the greatest amount of thiaminase activity in our studies were gizzard shad and spottail shiner (Table 1). Gnaedinger and Krzeczowski (1966) reported thiaminase activity in both of these species. It is interesting to note that Gnaedinger and Krzeczowski (1966) found that spottail shiner thiaminase activity was significantly greater than thiaminase activity in alewives also taken from Lake Michigan. Although the exact values for thiaminase activity cannot be compared directly between our studies and Gnaedinger and Krzeczowski's (1966) because of method dif-

ferences, the relative differences between thiaminase activity in spottail shiners and alewives were the same in both studies. Gizzard shad had similar amounts of thiaminase activity as spottail shiners in our studies (Table 1), but were reported to have one-tenth the activity of spottail shiners in Gnaedinger and Krzeczowski (1966). However, the source of gizzard shad was Lake Erie in their study, and lake-specific factors appear to influence thiaminase activity (Fitzsimons et al. 2005, this issue). The round goby analyzed in our studies contained only small amounts of thiaminase activity that were just near the level of detection for the assay. No previous information was available for this species. In summary, the two species with the greatest amounts of thiaminase activity, spottail shiners and gizzard shad, are not major components of the diets of lake trout or other salmonines in Lake Michigan (Miller and Holey 1992) and, as such, are not thought to be significant factors in the development of thiamine deficiency in Lake Michigan salmonines. The low thiaminase activity measured in yellow perch, ninespine stickleback, round goby, deepwater sculpin, and bloater suggests that these species are probably unable to contribute to thiamine deficiencies of salmonines in the Great Lakes.

Alewife, an introduced species in the Lake Michigan ecosystem, had large and variable amounts of thiaminase activity. Conversely, the variation in thiaminase activity measured in rainbow smelt was not significant among locations, seasons, or years of collection (GLM; Figure 2). Alewife and rainbow smelt from Lake Michigan were known to contain appreciable amounts of thiaminase activity (Deutsch and Hasler 1943; Neilands 1947; Gnaedinger and Krzeczowski 1966; Ji and Adelman 1998). The trends in thiaminase activity that we observed in our studies in Lake Michigan are not consistent with Ji and Adelman's (1998) report of thiaminase in the Great Lakes. In particular, we observed thiaminase activity in alewife to always be greater than thiaminase activity in rainbow smelt (Table 2). Ji and Adelman (1998) found rainbow smelt to contain more thiaminase activity than alewife (e.g., in the fall in Lake Michigan). At least part of these differences in our reports is probably a result of differences in methodologies related to the measurement of thiaminase activities in the forage species. Our protocols called for the addition of a base cofactor that was used during the measurement of the enzymatic reaction (Zajicek et al. 2005), while Ji and Adelman's (1998) procedures did not use a cofactor

during the measurement of the enzymatic activity. We used the cofactor in our assay to optimize conditions for the enzymatic reaction. It is known that thiaminase I activity *in situ* uses one of several base cofactors (Wittliff and Airth 1970); however, the amounts of these cofactors present at the site of the enzyme is not understood (Sato et al. 1994). Thus, our reports of enzymatic activities of thiaminase in forage fishes represent an optimal condition for the enzyme. We felt that understanding thiaminase activity under optimal, standardized conditions for the base cofactor in the forage fish samples would provide the best understanding of the enzymatic activity possible in the forage fishes. The values we report for thiaminase activities in alewife and rainbow smelt were significantly greater than Ji and Adelman (1998) reported. The ideal situation would be to have known amounts of the correct bases present at the site of the enzyme *in situ*, but this requires the knowledge of the dynamics of the thiaminase enzyme under intracellular and extracellular conditions encountered in the forage fishes and during digestion of the fishes. We simply do not have these exact conditions. Thus, providing optimal conditions for the enzyme will let us understand the full potential of the thiaminase present. Clearly, thiaminase activity is present in significant amounts in both rainbow smelt and alewife of Lake Michigan. The fact that alewife and rainbow smelt comprise a large portion of the lake trout diet in Lake Michigan (Miller and Holey 1992) combined with the elevated thiaminase activity observed in these two species suggests that these species are key causative factors in the onset and development of thiamine deficiency in Lake Michigan.

Thiaminase activity in alewife was positively correlated with fish size in the summer and fall, but this relationship was not found with rainbow smelt in our studies. The relationship of thiaminase with alewife size might help in the development of predictive models of thiamine deficiency; however, the seasonal effects must be considered and the strength of the relationship of thiaminase with size was not very strong. Fitzsimons et al. (2005) did not find a relationship among size and thiaminase in alewives collected from New York's Finger Lakes. The reasons for these differences are not known. Diets may influence thiaminase activity in alewife and other species. Alewife feed mainly on zooplankton (Janssen and Brandt 1980) and, thus, may be more susceptible to changes in primary producers than rainbow smelt, for example, which are more piscivorous (Brandt and

Madon 1986). The fact that rainbow smelt tend to be more piscivorous may cause them to have reduced fluctuations in thiaminase activity. Again, these factors and relationships are simply not known. The variability of thiaminase in alewife, compared with the fairly constant amount of thiaminase observed in rainbow smelt across locations, seasons, and years of collection, offers an important opportunity to study the factors that might control thiaminase in these fishes. It is clear that the simple presence of a species in the diet is not enough to cause thiamine deficiency in salmonines.

The source of thiaminase in forage fishes appears to originate from bacteria in the gut of the forage species and from possible dietary sources, such as the Cyanobacteria blue-green algae. Evidence for a bacterial origin of thiaminase in fishes comes from the fact that (1) thiaminase has been identified and the gene has been cloned in *Bacillus thiaminolyticus* (recently reclassified as *Paenibacillus thiaminolyticus*; Abe et al. 1987); (2) this bacteria has been isolated and cultured from the alewife digestive tract (Honeyfield et al. 2002); (3) the distribution of thiaminase in fishes is consistent with locations that bacteria are present in fishes (Fujita 1954; Zajicek et al. 2005); and (4) the biochemical characteristics (including pH and temperature optima) of the thiaminase found in forage fishes, and in particular alewife from Lake Michigan, are consistent with a bacterial source (see Zajicek et al. 2005). The greatest amount of thiaminase activity in fish, including forage fishes of Lake Michigan, is found in the tissues of the spleen, intestine, and kidneys (Fujita 1954; Zajicek et al. 2005). If thiaminase activities in forage fishes of Lake Michigan were the result of bacterial sources, then the differences in thiaminase activities observed among species (Table 1) may, in part, be a result of species-specific physiological conditions of the fishes. There appear to be some consistencies in the presence or absence of thiaminase activity among species that have been examined over the years (see discussions above). Even when spatial and temporal differences are considered, species that have thiaminase activity tend to always have this activity, while those that do not have thiaminase activity consistently do not show thiamine-degrading ability. Thus, a certain degree of species-specific factors such as physiological conditions appear to be important, regardless of external environmental factors. Environmental factors (temperature, dissolved oxygen, etc.) and dietary factors (prey species, food quality

and quantity) affect both the microflora and the physiological conditions of the fish (Fänge and Grove 1979). Thus, changes in physiological conditions in alewife may well affect growth characteristics of *Paenibacillus thiaminolyticus* (or other relevant microflora) and, in turn, affect the activity of thiaminase present in the alewife. However, essential nutrient requirements and optimal growth conditions are not known for these bacteria. A clear research need is to better understand nutritional and environmental factors that influence or limit the growth of microflora in alewife and, possibly, rainbow smelt. The ecological implications of thiaminase-producing bacteria in forage fishes are truly an intriguing question.

Another possible source of thiaminase and variation in thiaminase in the Great Lakes is blue-green algae. They are known to produce thiaminase (Fujita 1954), but not much is known about the characteristics of blue-green algae-derived protein. Arsan (1970) measured thiaminase in some freshwater species of fish and noted seasonal fluctuations. Thiaminase activities in the liver and intestines of all species in their studies were greatest in summer-caught fish organs. The author attributed the seasonal differences to blue-green algae-derived thiaminase from summer blooms. We found thiaminase activities to be smallest in alewives caught in the summer and greatest in alewives caught in the spring. It is certainly conceivable that differences in the limnology and subsequent ecology of the different lakes could result in different control mechanisms and sources of thiaminase in forage fishes (Fitzsimons et al. 2005). An evaluation of the enzymatic nature of blue-green algae-derived thiaminase and development of reagents for each type of thiaminase will provide tools to answer some of our challenging questions surrounding the role and origins of thiaminase in thiamine-deficient Great Lakes salmonines.

Conclusions

Evidence from our study is consistent with the notion that thiaminase activity in the major forage fish species is a key causative factor in the development of thiamine deficiency and subsequent EMS of Lake Michigan salmonines. The thiamine content in these same forage fish species was not low enough to be considered a prominent factor of this disease. There was some variation in the total thiamine content of alewife and rainbow smelt across seasons, locations, and years of collection, but the mean values never dropped below

dietary requirements for salmonines. Thiaminase activity was elevated in the most prominent forage fish species in the diets of salmonines, alewives, and rainbow smelt. Thiaminase was variable in alewife but fairly constant in rainbow smelt. The variation in thiaminase of alewife was not predicted simply by regional area of collection or the size of the fish. Thus, a more complex understanding of the factors that control thiaminase activity is required to make predictions about the impacts of forage fish thiaminase activity on salmonine populations and EMS.

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