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[Article]

Evaluating the Effect of Stressors on Thiaminase Activity in Alewife

JESSE M. LEPAK* AND CLIFFORD E. KRAFT
Department of Natural Resources, Cornell University, Ithaca, New York 14853-3001, USA

DALE C. HONEYFIELD
U.S. Geological Survey, Leetown Science Center, Northern Appalachian Research Laboratory, 176 Straight Run Road, Wellsboro, Pennsylvania 16901, USA

SCOTT B. BROWN[1]
Environment Canada, National Water Institute, 867 Lakeshore Road, Burlington, Ontario L7R 4A6, Canada

Abstract.—No consistent explanation has been found for the variability in the thiaminase activity of alewives *Alosa pseudoharengus* despite the role of alewife thiaminase in large-scale salmonine mortality in the Laurentian Great Lakes. We conducted experiments to evaluate the effect of two stressors, reduced salt content in the water and food limitation, on alewife thiaminase activity. Alewives were subjected to treatments in replicated tanks in which conductivity was lowered (<100 μS/cm) for 8 d and feeding was limited for 39 d. Circulating white blood cells, plasma cortisol, plasma glucose, and whole-body thiaminase were measured in individual alewives to assess their response to these experimental treatments. Alewives from the controls had significantly larger numbers of circulating white blood cells than those in the salt-reduced and food-limited treatments (24,000 and 19,000 cells/μL and 11,000 and 9,000 cells/μL for alewives from the two control and salt-reduced treatment tanks, respectively, and 34,000 and 30,000 cells/μL and 21,000 and 16,000 cells/μL for alewives from the two control and food-limited treatment tanks). No significant differences in alewife thiaminase activity were found between treatment fish and their controls. The mean thiaminase activity in the alewives studied increased from 6,900 to 16,000 pmol $\cdot g^{-1} \cdot min^{-1}$ from the time of their collection in Cayuga Lake to the start of laboratory experiments 1.5–2.5 years later; the latter value was more than twice that of previously reported levels of thiaminase activity from alewives collected in the wild. These data suggest that the variability in alewife thiaminase is not related to stress from salt reduction or food limitation, but laboratory holding conditions significantly increased thiaminase through a mechanism not evaluated by our experimental treatments.

Alewives *Alosa pseudoharengus* have been the most important forage fish in the Great Lakes for over 40 years (Madenjian et al. 2002; Mills et al. 2003; Dobiesz et al. 2005). Following alewife introductions and subsequent proliferation, Great Lakes food webs have been completely altered such that salmonine predators rely primarily on alewives as prey (Jude et al. 1987; Lantry 2001; Madenjian et al. 2002; Mills et al. 2003). Over the past three decades, a thiamine-deficiency-related reproductive failure (early mortality syndrome [EMS]) has been observed in valuable Great Lakes fishes, including lake trout *Salvelinus namaycush* and Atlantic salmon *Salmo salar* (McDonald et al. 1998; Honeyfield et al. 2005a). High levels of a thiamine-destroying enzyme, thiaminase, have been found in alewives (Fitzsimons et al. 2005; Tillitt et al. 2005), and thiaminase is responsible for EMS in salmon and trout that typically prey on alewives (Fitzsimons et al. 1999). Characteristically, offspring of salmonine fishes susceptible to EMS die shortly after hatching, but fry from identical egg sources survive and exhibit normal behavior when treated with thiamine (Fitzsimons and Brown 1998). The mechanism behind the expression of thiaminase in the alewife is unknown, and no single factor has consistently explained observed variability in thiaminase activity, though high levels of thiaminase have been associated with algae blooms (Burkholder 1998; McDonald et al. 1998; Fitzsimons et al. 1999), alewife condition, seasons, and location (Fitzsimons et al. 2005; Tillitt et al. 2005).

Honeyfield et al. (2002) isolated thiaminase-positive bacteria (*Paenibacillus thiaminolyticus* and other closely related bacteria) from alewife viscera, and alterations in diet levels of bacterially produced thiaminase have been used to demonstrate EMS in laboratory experiments with lake trout (Honeyfield et al. 2005b). In situations in which thiaminolytic bacteria

* Corresponding author: jml78@cornell.edu
[1] Deceased.

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are the source of thiaminase activity in alewives, changes in physiological conditions, such as those resulting from environmental or other sources of stress, that influence the growth characteristics of these bacteria can be expected to affect thiaminase activity (Tillitt et al. 2005). Laboratory studies have shown that one response of fish to chronic stress is a reduction in number of circulating white blood cells, which play a role in suppressing bacteria (Pickering 1984; Barton et al. 1987). The interaction between the clupeid immune system and internal bacterial communities is not understood, though changes in circulating white blood cells have been shown to serve as indicators of an immune response to stressful stimuli in other fishes (Iwama and Nakanishi 1996). Limited blood work was conducted on alewives in the late 1970s in a study of a red blood cell infection called piscine erythrocytic necrosis (PEN) (Sherburne 1977). Since that time hematological characteristics of the alewife have remained largely unexplored.

The influence of stress on alewives has been of interest since massive alewife die-offs were observed throughout the Great Lakes in the 1960s, and a limited number of early research efforts evaluated the physiological changes related to alewife mortality (Stanley and Colby 1971; Colby 1973). Subsequent studies suggested that several stressful factors (e.g., osmoregulatory alteration by freshwater and low food availability) could contribute to alewife mortality (Mills et al. 2003; Snyder and Hennessey 2003). The stress response in fish is linked to increased circulating plasma cortisol concentrations (Barton and Iwama 1991; Gamperl et al. 1994; Mommsen et al. 1999), which subsequently leads to immunosuppression when stressful conditions are chronic (i.e., occurring on the order of days to weeks or more; Mommsen et al. 1999). Intensive performance and mortality studies on fish have been conducted to examine the influence of various environmental conditions, including salinity and food availability, upon cortisol secretion (Barton and Iwama 1991; Mommsen et al. 1999). Most of these studies were conducted on salmonine fishes, and only two studies have evaluated cortisol levels in clupeid fishes, none of which included alewife (Davis and Parker 1986; Shrimpton et al. 2001). Thus, no information regarding alewife plasma cortisol or glucose levels is available from previous studies, and the effect of salt reduction and food limitation on alewife circulating white blood cells and thiaminase has not been evaluated.

Given our knowledge of the association between thiaminolytic bacteria and thiaminase in the alewife, we expected that environmental stressors capable of influencing alewife mortality and other physiological characteristics would also influence alewife thiaminase activity. Indirect effects associated with alewife stress could alter thiaminase activity, possibly through a mechanism whereby thiaminase is produced more efficiently, thiaminolytic bacteria proliferate, or both. Appropriate genetic tools have not yet been applied to evaluate thiaminolytic bacterial communities or the expression of the thiaminase gene.

Low salinity and food limitation were selected as treatments in this experiment to examine their effects on thiaminase in alewives. We hypothesized that these treatments would result in lower circulating white blood cells, ultimately leading to an increase in thiaminase activity. Alewife plasma cortisol and glucose levels were also measured to evaluate whether acute factors could contribute to differences in thiaminase activity.

Methods

Alewives were collected in the spring of 2003 (food-limited trials) and 2004 (salt-reduced trials) from Cayuga Lake, New York, and then transported by truck to the U.S. Geological Survey's Northern Appalachian Research Facility in Wellsboro, Pennsylvania. Whole alewives ($N = 10$) from the 2004 sampling period were flash-frozen on dry ice and sent to the Canadian Center for Inland Waters (CCIW), Burlington, Ontario, for thiaminase analysis as described later to evaluate initial thiaminase activity. An additional 30 alewives collected in 2004 were weighed and then dried for a minimum of 120 h to evaluate their water content as an indicator of condition. Before the start of the experiments, alewives were held at densities of approximately 50–100 fish/m^3 in 2-m-diameter, circular, flow-through tanks (4-m^3 volume) supplied with well water. Commercial trout feed was provided to alewives once each hour from 0800 to 1600 daily with automated feeders until experimental trials began in spring 2005. Alewives were transferred from holding tanks to identical experimental tanks in groups of 22 (12 fish for analysis and an additional 10 fish to be used in the event of mortality). Alewives from the same holding tank were used for each treatment and associated control to ensure that fish within all comparisons were held and transferred under similar conditions before the experiments. Conductivity within the holding tanks was maintained above 300 μS/cm by adding artificially produced saltwater (water to which sodium chloride had been added) to the incoming well water; these salinity levels were regulated individually for each tank by a metered pump. Water temperatures were maintained at approximately 13.5°C throughout the experiment by means of heaters in each tank. All holding tanks were slowly adjusted to these conditions

to ensure that fish experienced as little stress as possible before the experiments. A minimum of 72 h was allowed for acclimation after the transfer process, which is a typical amount of time required for physiological "resting" plasma cortisol levels to return to normal after tank transfer and handling stress (Pickering 1984). Fish in each tank were randomly assigned to a control or treatment group. Both treatment and control groups were replicated twice, resulting in a total of four experimental tanks containing 22 alewives each for both the salt reduction and food limitation trials (176 alewives total).

At the completion of each trial, 12 alewives were removed individually from each tank using a small dip net with as little disturbance as possible to the remaining fish. Alewives were collected by allowing a single fish to swim into the dip net under its own volition, after which it was slowly removed from the tank. The netting procedure was conducted under low light conditions to further minimize stress to alewives during sampling. Each alewife was heavily anesthetized with tricaine methanesulfonate ([MS-222], 125 mg/L) and subsequently killed. Blood (~650 μL) was extracted from the caudal vessel in the hemal arch of each captured alewife using heparinized syringes, after which blood was centrifuged at 20,800 × gravity for 5 min. Plasma was decanted from the blood samples and stored at −20°C until plasma cortisol and glucose analyses were conducted. A solid-phase radioimmunoassay was used to measure plasma cortisol (Davis and Parker 1986), and a double enzymatic procedure was used to measure glucose (Sigma Diagnostic, St. Louis, Missouri) at the College of Veterinary Medicine, Cornell University. Duplicate measurements were taken for each individual sample. If the duplicate measurements did not correspond or the means of these measurements were outside of the acceptable range relative to other samples in the experiment (based on the discretion of laboratory personnel experienced in measuring cortisol and glucose), the sample was run in triplicate. All blood samples were collected within 4 min of netting each individual fish.

An additional droplet of blood from each alewife netted was collected for white blood cell differential counts. The droplet was used to immediately prepare blood smears on glass slides that were dried and subsequently fixed and stained using a three-step Diff-Quik kit (Sigma-Aldrich Chemical, St. Louis, Missouri). We performed white blood cell differential counts by locating monolayered cell regions on each slide (using 1,000× magnification), then identifying the first 200 white blood cells that were observed within each blood smear. These analyses were conducted by a single trained individual for standardization. The resulting data were used to generate ratios of lymphocytes, neutrophils, monocytes, and other granulocytes. Owing to their relative scarcity, eosinophils and basophils were combined into a single category, referred to as "other granulocytes." A white blood cell count was determined by averaging the number of white blood cells within 10 high-power fields (400× magnification) in a monolayer portion of each slide, then multiplying this value by 2,000 to arrive at a cell count quantified as number per microliter (Campbell 1994a, 1994b). The differential ratios and white blood cell count data were combined to obtain a total count for each cell type. These estimated values for each cell type assumed that each slide was prepared in a similar fashion (i.e., similar pressure was used for each smear) and that the blood was of similar viscosity. Cells were not counted at slide locations that included clumped regions of cells. To maintain consistency in the white blood cell count estimates, these counts were also conducted by a single trained individual.

Thiaminase analyses were conducted on each of the 12 captured fish per tank. Thiaminase activity ($pmol \cdot g^{-1} \cdot min^{-1}$) was measured on individual, whole, homogenized fish at CCIW using the radiometric procedure described by Zajicek et al. (2005). All 48 alewives sent for thiaminase analysis were flash-frozen on dry ice immediately after collection and blood sampling before shipping. The remaining alewives (i.e., those not evaluated for thiaminase) from the original 22 individuals in each tank were weighed, and whole-body water content (an indicator of condition, see Flath and Diana 1985) was measured by drying each fish in a drying oven for a minimum of 120 h at 60°C.

Experimental treatment conditions.—Salinity treatments were initiated March 21, 2005, and ended on March 28. Salinity levels in the two treatment tanks were lowered to less than 100 μS/cm to induce osmoregulatory stress, and all other conditions within treatment and control tanks remained as stated previously. Food limitation treatments began April 8 and ended on May 16. To represent low food availability as a form of stressor, feeding was stopped in the two treatment tanks; however, all other conditions within treatment and control tanks remained as stated previously. Both of these treatments were chosen because they had been shown previously to produce mortality in alewives held in captivity at the Northern Appalachian Research Laboratory (D. C. Honeyfield, unpublished data).

Netting stress trial.—A brief experiment was conducted to evaluate the assumption that the process of netting an individual alewife did not influence plasma cortisol levels in other fish in the tank.

Alewives were transferred from a holding tank to three additional tanks with similar conductivity, temperature, and feeding conditions in groups of 15 (45 fish total), after which these alewives were subsequently recaptured 72 h later. During this and other study experiments, fish were not chased and were only netted from tanks when they swam into the capture net on their own accord. After netting, alewives were sampled for plasma cortisol as described above. The only exception to this procedure was the 14th fish from tank 3 (see Results), which was inadvertently chased vigorously for over a minute and processed after a period of approximately 10 min. Aside from this exception, all other fish were captured and blood collection was completed in less than 4 min.

General stress trial.—To assess the ambient levels of alewife plasma cortisol and glucose in a hatchery setting, we conducted an exploratory experiment to determine plasma cortisol and glucose in stressed and unstressed fish. Alewives collected from Cayuga Lake in spring 2003 and held in captivity until April 2005 were used to evaluate whether stress in the hatchery had limited their ability to respond to stressful conditions. Fifteen alewives were transferred to a holding tank. Following a 72-h acclimation period, five alewives were taken directly from the tank and blood was immediately collected for plasma cortisol and glucose analysis as described previously. The remaining alewives ($N = 10$) were also captured, placed in a bucket with about 2 L of water, and chased vigorously with a net for 5 min. These alewives were left in the bucket for an additional 10 min after which sampling for plasma cortisol and glucose was conducted as described previously.

Statistical analyses.—The relationships between alewife thiaminase and circulating white blood cells, plasma cortisol, and plasma glucose were evaluated by means of linear regression in SAS (SAS Institute, Cary, North Carolina). Mixed-model analyses with tank as a random effect (reflecting the random assignments of fish and treatments to individual tanks) and treatment as a fixed effect were conducted with the PROC_-MIXED procedure using SAS to test for treatment effects on alewife thiaminase, white blood cell counts, plasma cortisol, and plasma glucose. The PROC MIX procedure in SAS is a generalization of the standard linear model procedure that specifically addresses individual measurements in experimental units that can be grouped or clustered (in this case, tanks) to account for random variability in these experimental units. The Satterthwaite method was used to calculate the degrees of freedom (reported in the tables summarizing these analyses). Simple linear regression was conducted using SAS to examine the relationship between alewife plasma cortisol and glucose as a function of the time after the first alewife was sampled in the netting stress trials. Two-sample *t*-tests were conducted using SAS to test for the effect of experimental treatments on plasma cortisol and glucose levels within alewives from the general stress trial.

Results

The initial thiaminase activity of the 10 alewives collected from Cayuga Lake in spring 2004 was 6,900 $\pm$ 2,800 pmol$\cdot g^{-1}\cdot min^{-1}$ (mean $\pm$ SD). The overall value of alewife thiaminase activity for all 96 study fish at the completion of our experiments (ranging from 1.5 to 2.5 years after fish were captured in the wild) was 16,000 $\pm$ 5,900 pmol$\cdot g^{-1}\cdot min^{-1}$, which was a substantial increase relative to the initial activity measured when alewives were first brought to the laboratory. No significant difference was found between the mean thiaminase levels in alewives from the salt-reduced and food-limited treatment tanks and those from the respective control tanks (Table 1). Regression analyses showed no significant relationship between alewife circulating white blood cells, plasma cortisol, or plasma glucose and the dependent variable, thiaminase activity, within all treatment and control fish (Table 2). The water content of the 30 fish sampled from Cayuga Lake in spring 2004 was 72.0 $\pm$ 1.6%; the water content of alewives at the completion of the experiments was 56.3 $\pm$ 3.5% for control and treatment fish in the salt-reduced experiment ($N = 40$) and 51.1 $\pm$ 6.9% for control and treatment fish in the food-limited experiment ($N = 37$).

Total white blood cell counts were lower in both treatments (salt reduced and food limited) than in their associated controls (Table 1). Alewife plasma cortisol was significantly higher in fish from control tanks than in fish from salt-reduced treatment tanks; alewife plasma cortisol was not significantly different in food control tanks than in food-limited tanks (Table 1). Alewife plasma glucose levels in control tanks were not significantly different from their respective salt-reduced and food-limited treatments (Table 1). Seven measurements of plasma cortisol and glucose were tested in triplicate based on disparity in duplicate measurements or atypical values based on the discretion of laboratory personnel. Three of these repeat analyses led to results similar to the initial results despite exceptionally high mean values; therefore, these values were included in subsequent statistical analyses. Four of these analyses conflicted with the initial results, that is, the third value was not comparable to either of the first two measurements. Therefore, the values from these fish were excluded from further analyses (one measurement of plasma

TABLE 1.—Mean ± SD alewife thiaminase, total white blood cell count, plasma cortisol, and glucose and mixed-model comparisons between salt-reduced and food-limited treatments and controls. Except where indicated otherwise, 12 fish were tested. The numerator degrees of freedom = 1 for all comparisons. The denominator degrees of freedom = 45 for the plasma cortisol comparison for the salt-limited treatment and 46 for the thiaminase comparison for the salt-limited treatment and the total white blood cell comparison for the food-limited treatment; for all other comparisons, the denominator degrees of freedom = 2.

Treatment	Replicate	Thiaminase (pmol·g^{-1}·min^{-1})	Total white blood cell count (number/μL)	Plasma cortisol (μg/dL)	Plasma glucose (mg/dL)
		Reduced-salt experiments			
Control	1	18,000 ± 6,000	24,000 ± 9,400	18.7 ± 14.9	109.0 ± 58.3
	2	16,000 ± 3,900	19,000 ± 6,200	15.1 ± 8.7	53.4 ± 15.8
Salt limited	1	14,000 ± 5,900	11,000 ± 3,800	8.1 ± 3.5[a]	116.4 ± 35.7
	2	15,000 ± 3,500	9,000 ± 4,600	7.8 ± 8.1	115.6 ± 44.8
F-value		3.79	17.34	10.20	1.57
P-value		0.06	0.05	<0.01	0.34
		Food limitation experiments			
Control	1	14,000 ± 4,500	34,000 ± 12,000	8.2 ± 6.2	91 ± 31.7
	2	17,000 ± 7,300	30,000 ± 15,000	21.4 ± 14.3[a]	126.3 ± 49.5
Food limited	1	21,000 ± 8,200	21,000 ± 11,000	6.7 ± 10.7	56.7 ± 27.3
	2	17,000 ± 4,400	16,000 ± 12,000	9.0 ± 13.9[a]	108.4 ± 72.7[a]
F-value		1.53	14.68	1.05	0.71
P-value		0.34	<0.01	0.41	0.49

[a] Eleven fish.

cortisol was excluded from one salt-reduced and one food-limited treatment and one food-control group, and one measurement of plasma glucose was excluded from one food-limited treatment).

Alewife lymphocyte counts were significantly greater in alewives from control tanks than in those from salt-reduced treatment tanks. Monocyte and other granulocyte counts were higher in fish from control tanks than those in food-limited tanks. All other comparisons of differential white blood cell counts were not significant (Table 3).

No evidence was found for an influence of the process of netting fish on plasma cortisol in other alewives within the same tank, with the exception of a single alewife that was inadvertently chased during capture (Figure 1). The results from this trial are shown in Figure 1 in the order in which alewives were captured and processed. The 14th fish from tank 3, which was exposed to substantial handling stress and not processed until approximately 10 min after its first exposure to being chased by a net, had elevated plasma cortisol. No significant increases or decreases ($P > 0.05$ in all linear regression analyses) in alewife plasma cortisol or glucose within any of the replicate tanks were found in analyses that both included and excluded this fish from the regression analyses. Based on this evidence that plasma cortisol did not respond to the netting procedure, we consider longer-term indicators of stress (e.g., white blood cell counts) to have also been unaffected by the netting procedure.

Fish that were vigorously chased showed significantly higher plasma cortisol levels than the controls, but the plasma glucose levels of these alewives were unaffected (Table 4).

Discussion

The experimental treatment conditions in this study resulted in lower circulating total white blood cell counts in alewives exposed to the treatments than in their respective controls. This response has been shown in previous studies of fishes to be an indicator of an altered immune response (Pickering 1984; Barton et al. 1987). However, alewife thiaminase levels did not increase in response to these experimental treatments. In addition, no significant relationship was found between alewife plasma cortisol and glucose levels,

TABLE 2.—Linear regression statistics for the relationships between the dependent variable, alewife thiaminase levels (pmol·g^{-1}·min^{-1}), in the salt-reduced and food-limited treatments and controls and total white blood cell counts, plasma cortisol, and glucose.

	Total white blood cell count (number/μL)			Plasma cortisol (μg/dL)			Plasma glucose (mg/dL)		
Experiment	r^2	*P*	*N*	r^2	*P*	*N*	r^2	*P*	*N*
Salinity	0.01	0.46	48	0.02	0.29	47	0.04	0.18	48
Feeding	<0.01	0.82	48	0.01	0.49	46	0.05	0.12	47

TABLE 3.—Mean ± SD alewife lymphocyte, neutrophil, monocyte, and other granulocyte counts (cells/μL) and mixed-model comparisons between salt-reduced and food-limited treatments and controls. The number of fish tested is 12 in each case. The numerator degrees of freedom = 1 for all comparisons. The denominator degrees of freedom = 46 for the monocyte comparison for the food-limited treatment and the other granulocyte comparison for the salt-reduced and food-limited treatments; for all other comparisons, the denominator degrees of freedom = 2.

Treatment	Replicate	Lymphocytes	Neutrophils	Monocytes	Other granulocytes
Reduced-salt experiments					
Control	1	17,000 ± 7,400	5,300 ± 6,000	2,000 ± 1,400	150 ± 200
	2	16,000 ± 4,900	2,200 ± 3,500	1,200 ± 530	67 ± 100
Salt limited	1	7,100 ± 3,900	2,600 ± 2,400	1,400 ± 840	130 ± 230
	2	6,300 ± 3,400	1,700 ± 1,500	860 ± 520	120 ± 120
F-value		42.76	1.00	0.89	0.05
P-value		<0.01	0.42	0.44	0.82
Food limitation experiments					
Control	1	29,000 ± 10,000	2,500 ± 1,500	1,300 ± 660	320 ± 580
	2	22,000 ± 13,000	7,200 ± 6,800	1,500 ± 1,100	230 ± 230
Food limited	1	18,000 ± 11,000	2,200 ± 2,000	650 ± 610	76 ± 120
	2	13,000 ± 12,000	2,800 ± 2,300	890 ± 620	82 ± 120
F-value		4.88	0.98	7.24	4.45
P-value		0.16	0.43	<0.01	0.04

both of which have been reported as primary and secondary stress indicators (Barton 1997), and alewife thiaminase activity. The overall alewife response to experimental treatments in our study suggests that circulating white blood cells and plasma cortisol and glucose levels were not associated with alewife thiaminase activity. However, the mean alewife thiaminase activities observed in this experiment were more than twice as high (16,000 pmol·g^{-1}·min^{-1}) as those observed in alewives collected from other lakes in the Great Lakes basin (1,700 to ~6,000 pmol·g^{-1}·min^{-1}), which have shown substantial variation both seasonally and across systems (Fitzsimons et al. 2005; Tillitt et al. 2005).

The total white blood cell counts of fish within control tanks were higher than those of fish in treatment tanks, and alewife lymphocytes were significantly greater in number in the salt-reduced controls than in their respective treatments. The lower counts of white blood cells observed in alewives from the salt-reduced treatments were similar to those observed in other fish species exposed to chronic stress. For example, striped bass *Morone saxatilis* were found to have significantly lower white blood cell counts (~20,000 total white blood cells/μL versus ~60,000 total white blood cells/μL, respectively) in laboratory experiments after a 6-week exposure to cold (10°C) versus warm (18°, 24°, and 29°C) water temperatures

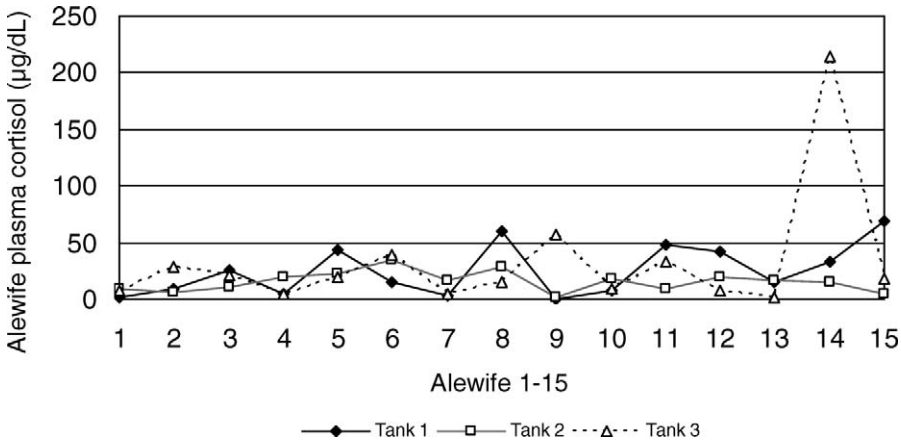

FIGURE 1.—Alewife plasma cortisol levels from the netting stress trial. The numbers of the replicate tanks (1–3) are based on the order in which they were randomly sampled, as are the numbers of the fish (1–15) in each tank. There were no significant trends in plasma cortisol with respect to sampling time whether or not we included the data for fish 14 from tank 3 (which was chased for an extended period owing to poor netting and then processed approximately 10 min after the initial netting attempt).

TABLE 4.—Mean ± SD alewife plasma cortisol and glucose and results from two-sample *t*-tests comparing control and deliberately stressed fish. The letter *N* represents the number of fish tested; the degrees of freedom for both tests is 13.

Treatment	*N*	Plasma cortisol (μg/dL)	Plasma glucose (mg/dL)
Control	5	2.0 ± 3.5	85.6 ± 25.1
Vigorous chasing	10	43.9 ± 12.9	68.1 ± 3.6
t		9.58	1.55
P-value		<0.01	0.20

intended to induce chronic stress (Hrubec et al. 1997). In our study, alewife monocyte and other granulocyte counts were significantly higher in control fish than in those in treatment tanks in the food-limited trial. Our results indicate that salt reduction and food limitation resulted, to different degrees, in lower circulating white blood cell counts. However, the absence of a concurrent thiaminase response provides no support for the hypothesis that thiaminolytic bacteria or any other internal processes responsible for producing thiaminase were influenced by the alewife immune system response observed in these experiments.

The significantly lower plasma cortisol levels observed in the salt-reduced experimental treatment were unexpected. This observed response may have resulted from an extended exposure to stressful conditions (e.g., an initial cortisol increase in response to the salt reduction treatment might have been subsequently followed by a decline in cortisol). Hontela et al. (1992) found that fish exposed to prolonged stressful conditions from a polluted environment exhibited an impaired response to immediate stress and cortisol levels within these fish did not increase in response to stress from capture and handling. Those investigators also observed that the pituitary corticotropes (cells in the anterior pituitary that produce the adrenocorticotropic hormone responsible for stimulating the adrenal gland to produce cortisol) were atrophied in their study fish. A similar level of impairment in alewives within our salt-reduced treatment may have influenced the observed alewife plasma cortisol response. It is also possible that the exposure to low salt levels may have influenced other physiological processes not evaluated in this study that also influenced the cortisol response to stress. However, plasma cortisol levels observed in alewives within the salt-reduced treatment tanks remained within a similar range of values as those measured in other groups of alewives in our experiments.

Plasma glucose levels were not significantly different in alewives from control and treatment tanks in the salt-reduced and food-limited trials. Environmental stressors, including osmoregulatory challenges, are known to produce increases in plasma glucose levels in several species of fish (Barton and Iwama 1991; Mommsen et al. 1999), whereas starvation decreases the level of plasma glucose in fish as a result of decreased concentrations of blood metabolites from the lack of diet-supplied glucose (Zammit and Newsholme 1979; Black and Love 1986). The overall mean concentrations of alewife plasma glucose in the salinity-reduced and food-limited trials relative to their respective controls showed trends similar to these previous studies, but these trends were not significant. Additional data regarding the short-term response of alewife plasma glucose levels to experimental treatments were not collected during the course of our experiments, which lasted from 7 (salt-reduced treatment experiment) to 38 d (food-limited treatment experiment).

The timing of the rapid increase in alewife plasma cortisol (approximately 15 min) observed in response to vigorous chasing within the general stress trial corresponded with expectations based on previous studies, as did the lack of a rapid plasma glucose response. The significant observed increase in plasma cortisol levels following induced stress from chasing fish with a net confirms that alewives used in this experiment were capable of responding to stress. We also consider it important to note that the alewife densities in the tanks during the experimental trials were maintained at a level approximately one order of magnitude lower than that in the holding tanks before the start of the experiment to ensure that any induced stress resulted only from treatment effects. The data presented provide new information regarding white blood cell counts, plasma cortisol, and plasma glucose levels in alewives and thus represent a contribution to current knowledge of these factors in clupeid fishes.

Although significant differences were found in circulating total white blood cell counts in response to experimental treatments, our results suggest that these treatments did not influence alewife thiaminase activity in our study fish. It is possible that thiaminolytic bacteria associated with alewife viscera (Honeyfield et al. 2002) might respond to changes in internal physiological conditions in alewives that were not associated with factors evaluated in this study or that thiaminase activity in alewives could be controlled by some other factor or combination of factors. Furthermore, the alewives in this study were not exposed to any known external sources of thiaminase found in natural aquatic systems (e.g., cyanobacteria and zooplankton), and their artificial feed was thermally treated to denature any dietary sources of thiaminase, thereby eliminating food as a thiaminase source while

these fish were maintained in the laboratory. Therefore, we consider the following observations in our study to be of particular importance: (1) the mean value of thiaminase activity within our study alewives (16,000 $pmol \cdot g^{-1} \cdot min^{-1}$) was more than two times the previously published mean values for alewives collected from other North American lakes (Fitzsimons et al. 2005) and (2) the mean thiaminase levels for the alewives in our experiments increased from 6,900 to 16,000 $pmol \cdot g^{-1} \cdot min^{-1}$ after 1.5 to 2.5 years of laboratory rearing. These findings suggest that additional research is warranted to investigate changes in physiological responses and thiaminase activity within captive alewives, beginning at the point of capture and continuing through an extended period in captivity.

We discount the possibility that stress during captivity resulted in the observed two-fold increase in thiaminase activity based on two considerations. First, fewer than 10 alewives died during the 12 months before the initiation of the experiments; lack of mortality generally provides an indication of satisfactory rearing conditions. Second, alewives grew rapidly during captivity and exhibited exceptional body condition, measured as percent water content, at the start of the experiments (51–56%) versus at the time of their capture in the wild (72%). Whole-body lipid content is inversely related to body water content, and the water content of captive alewives in these experiments was lower than that of any fish previously reported (Hartman and Brandt 1995). It is not credible to suggest that stressed fish could improve body condition and sustain low mortality for more than a year in captivity, particularly a species such as the alewife that is not readily maintained in laboratory conditions (Colby 1973).

We may summarize the results of our experiments as follows:

1. We found no evidence linking stress to thiaminase activity in the alewives subjected to our experimental treatments.
2. We found no evidence that the alewives in our experiments were chronically stressed by laboratory conditions, based on the fact that plasma cortisol levels increased as expected when a netting stressor was applied.
3. We observed lower white blood cell counts in the alewives subjected to experimental treatments designed to produce stress (reduced salinity and low food availability) than in control fish, and similar white blood cell changes have been reported in response to similar stresses in other studies.
4. The thiaminase levels in the alewives in our study increased in response to laboratory holding conditions, but through a mechanism that was not evaluated by the experimental treatments.

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