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Naturally Occurring Thiamine Deficiency Causing Reproductive Failure in Finger Lakes Atlantic Salmon and Great Lakes Lake Trout

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Abstract.—A maternally transmitted, noninfectious disease known as the Cayuga syndrome caused 100% mortality in larval offspring of wild-caught landlocked Atlantic salmon *Salmo salar* from several of New York's Finger Lakes. Survival of lake trout *Salvelinus namaycush* from Lakes Erie and Ontario was also impaired, but not until yolk absorption was nearly complete; moreover, mortality was greatly reduced relative to that of the salmon (range: 5–87%). Tissue concentrations of thiamine hydrochloride were severely reduced in these salmonid fish relative to unaffected control stocks. Afflicted Atlantic salmon treated with thiamine by yolk-sac injection or by bath immersion recovered completely from the Cayuga syndrome, as evidenced by the quantified reversal of abnormal swimming behaviors only 2 d after treatment and by the excellent survival (>95%) of the treated Atlantic salmon through 1.5 months of feeding. These data represent the first evidence of a vitamin deficiency causing the complete reproductive failure of an animal population in nature. These lethal vitamin deficiencies are presumably caused by a diet of alewives *Alosa pseudoharengus*, nonnative forage fishes of the herring family that exhibit high thiaminase activity.

The fisheries of New York's Finger Lakes and the Laurentian Great Lakes (Figure 1) have been greatly altered by overfishing, habitat destruction, pollution, and the introduction of nonnative species (Youngs and Oglesby 1972; Christie 1974; Webster 1982; Mills et al. 1993). Two salmonid species native to these regions, the Atlantic salmon *Salmo salar* and the lake trout *Salvelinus namaycush*, have been especially affected. The erection of mill dams and erosive agricultural practices blocked or degraded critical spawning habitat and

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led to the elimination of all endemic Atlantic salmon stocks in the Finger Lakes and their eastern Lake Ontario catchment by the late 1800s (Webster 1982). Likewise, lake trout stocks in most of the Great Lakes were nearly eliminated by 1950 through predation by nonnative sea lamprey *Petromyzon marinus* and through overfishing (Christie 1974; Hartman 1988).

Releases of yearling Atlantic salmon originating from Little Clear Pond (LC), the Adirondack progenitor stock for all lakes with landlocked populations of Atlantic salmon in New York State, failed to reestablish reproductively viable salmon in Cayuga (CL), Seneca (SL), and Keuka (KL) lakes. Atlantic salmon progeny from these three Finger Lakes are afflicted with the 'Cayuga syndrome,' a noninfectious disease that kills all yolk-sac fry, even under optimum incubation conditions (Fisher et al. 1995a). Similarly, numbers of naturally produced, hatchery-strain lake trout fry remain low to nonexistent in lakes Ontario (Marsden et al. 1988), Michigan (Jude et al. 1981), and Erie (Hartman 1988). Although numerous factors reduce the reproductive success of the lake trout in these Great Lakes (e.g., predation and habitat loss), a noninfectious 'swim-up syndrome' that kills first-feeding fry at the completion of yolk absorption undoubtedly contributes to their poor recruitment (Skea et al. 1985; Mac et al. 1985, 1993; Fitzsimons et al. 1995).

Although both the Cayuga and the swim-up syndromes were first observed in 1974 (Fisher et al. 1995a; Mac et al. 1985), the dissimilarities of these two conditions suggested different etiologies. The Cayuga syndrome killed all progeny from every female salmon examined; death occurred several weeks before complete yolk absorption; lesions such as congestion, hemorrhage, subcutaneous and retrobulbar edema, and yolk opacities were common; behavioral signs such as convulsive swimming, and abnormal phototaxis and thigmotaxis indicated a prominent neurological correlate to the disease (Fisher et al. 1995b); and no other salmonid species in the Finger Lakes were affected (Fisher et al. 1995a). Clinical signs of the swim-up syndrome contrasted greatly with those of the Cayuga syndrome (Mac et al. 1985; Mac and Edsall 1991; Fitzsimons et al. 1995): mortality of the lake trout rarely exceeded 40% and varied greatly between years and female parent, death occurred at yolk absorption or shortly thereafter, lesions were absent, behavioral signs were evidenced principally as a loss of equilibrium, and a similar swim-up syndrome was documented in other species such as coho salmon *Oncorhynchus kisutch* (Johnson and Pecor 1969), and rainbow trout *O. mykiss* (Skea et al. 1985).

Hypotheses to explain the reproductive failure of Great Lakes basin salmonids have focused heavily on the role of environmental contaminants as potential endocrine disrupters (Leatherland 1993). The high levels of halogenated aromatic hydrocarbons (HAH) detected in tissues of Great Lakes salmonids (Giesy et al. 1986; DeVault and Dunn 1989; Ankley et al. 1991; Whittle et al. 1992), and the extreme sensitivity of embryonic and newly hatched lake and rainbow trout to dioxins, polychlorinated biphenyls, and dibenzofurans (Spitsbergen et al. 1991; Walker and Peterson 1991) suggested that the chlorinated toxicants were responsible. Yet controlled exposures to such HAHs failed to reproduce the clinical signs of these syndromes in lake trout (Spitsbergen et al. 1991), rainbow trout (Walker and Peterson 1991), or LC Atlantic salmon (Fisher et al. 1993). Furthermore, field investigations did not consistently correlate egg residues of specific HAH congeners or total dioxin (i.e., 2,3,7,8-tetrachlorodibenzo-*p*-dioxin) equivalents to early life stage mortality (Skea et al. 1985; Williams and Giesy 1992; Mac et al. 1993; Fisher et al. 1993; Smith et al. 1994; Fitzsimons et al. 1995). Heavy metal and pesticide contamination were also considered in the etiologies of both syndromes, but no consistent correlations were found (Fisher 1995; Fitzsimons et al. 1995). Thus, although toxicants were strongly implicated in the reproductive failure of piscivorous mammals (Reijnders 1986; Wren 1991; Beland et al. 1993) and birds (Tillitt et al. 1992), their role in these Great Lakes basin piscine syndromes was unproven.

Evidence from epizootiological studies of the Finger Lakes strongly suggested that diet was involved: only those Finger Lakes populated with the nonnative alewife *Alosa pseudoharengus* had Atlantic salmon populations affected by the Cayuga syndrome (Fisher et al. 1995a; Figure 1). Similarly, alewives are the primary forage of lake trout in Lakes Michigan (Jude et al. 1987; Miller and Holey 1992) and Ontario (Elrod 1983; Brandt 1986). Given that the alewife has high thiaminase activity (Gnaedinger 1964) and that the neurological signs exhibited by the Atlantic salmon and lake trout suggested a thiamine (vitamin B-1) deficiency (Halver 1957), experiments were performed to determine the role of thiamine in these seemingly disparate syndromes.

In preliminary experiments, thiamine treatment

significantly reduced the mortality associated with the swim-up syndrome in Lake Ontario lake trout (Fitzsimons 1995). However, the syndrome was not completely eliminated, and thiamine status was not assessed; thus, whether a thiamine deficiency was the cause of the swim-up syndrome remained to be determined. Here we present results of thiamine therapy of Atlantic salmon afflicted with the Cayuga syndrome and report whole body thiamine concentrations from moribund Finger Lakes Atlantic salmon sac fry relative to the unaffected progenitor stock; thiamine levels in lake trout eggs from Lakes Ontario and Erie are also compared to those of a hatchery control stock. These data support the previously proposed relation between the early mortality syndromes of these species and a thiaminase-rich alewife diet (Fisher et al. 1995a; Fitzsimons et al. 1995).

Methods

Source of test fish and husbandry conditions.—Atlantic salmon sac fry used for these studies were hatched from eggs stripped from 1993 broodstock. Control broodstock from LC (N = 10) were captured by trap net and spawned on-site with the sperm of 3–5 males. These broodstock are principally fortified on a natural diet of rainbow smelt *Osmerus mordax*. The LC eggs were incubated through eye-up at the New York State Department of Environmental Conservation (NYSDEC) Adirondack Hatchery, Saranac Lake, New York, adjacent to LC. After eye-up, LC eggs were transported to aquaculture facilities at Cornell University and cultured as described (Fisher et al. 1995a). We captured broodstock from CL (N = 6), SL (N = 8), and KL (N = 1) by electroshocking in tributaries to these systems during the late October to mid-November spawning season (Fisher et al. 1995a). Eggs and resultant sac fry from these stocks were separated by female parent throughout incubation except when otherwise noted. Maintenance of all fish stocks was in accordance with institutional guidelines of Cornell University. All surviving fry from these studies were released into CL tributaries with the assistance of NYSDEC personnel about 1.5 months after feeding began.

Control lake trout eggs were acquired during November 1992 from hatchery broodstock (N = 7) that were cultured at the Ontario Ministry of Natural Resources research facility (Maple, Ontario; Figure 1). These broodstock were fed a commercial diet supplemented with thiamine. Experimental lake trout eggs from Lake Ontario stocks were acquired during October and November 1991 from broodstock captured by gill net near Fifty Point (N = 2) and Stony Island (N = 6). Experimental lake trout eggs from Lake Erie were acquired in early November 1992 from broodstock captured by trap net in Barcelona Harbor, New York. Eggs from all lake trout stocks were separated by female to assess swim-up syndrome mortality. Eggs from the control, Fifty Point, and Barcelona Harbor stocks were fertilized with the pooled sperm of about nine males. Eggs from each of the Stony Island females were fertilized with sperm of two or three males. After rinsing of the sperm, the eggs from each female were randomly placed in a numbered section of a horizontal flow raceway and incubated at 8°C at greater than 80% oxygen saturation. A single replicate of 200 eggs per female was used for the control and, Fifty Point and Barcelona Harbor stocks; three replicates of 200 eggs were monitored from each female of the Stony Island stock.

Dead eggs were picked every day and no prophylactic treatments were used during the incubation period. Fry exhibiting signs of the swim-up syndrome (e.g., loss of equilibrium, lying on their sides on the bottom of the tank, hyperexcitability) were removed. Previous observations had indicated that once fry were afflicted, they did not recover and eventually died. No feed was offered to swim-up fry because Fitzsimons et al. (1995) found that there was no significant decrease in swim-up syndrome mortality when fish were fed. Swim-up syndrome mortality was expressed as percent fry exhibiting clinical signs associated with the syndrome relative to the number of fry that hatched. Observations of swim-up syndrome mortality were concluded approximately 1 month after swim-up.

Vitamin therapy in Atlantic salmon sac fry.—The effectiveness of thiamine hydrochloride at treating the Cayuga syndrome was examined by microinjection and by aqueous exposure. Positive-control Atlantic salmon consisted of untreated, syndrome-afflicted sac fry from which previously reported mortality records were maintained by maternal and lake source (Fisher et al. 1995a). When appropriate, negative-control sac fry from the LC progenitor stock were included.

Vitamins E, C, A, and a multivitamin were also tested for their therapeutic efficacy at treating the Cayuga syndrome. Vitamins, solvents, and anesthetics were reagent grade from Sigma Chemicals (St. Louis, Missouri), except the multivitamin mixture (Your Life Multi-Vitamin, Leiner Health Products, Inc., Carson, California). Vitamins E

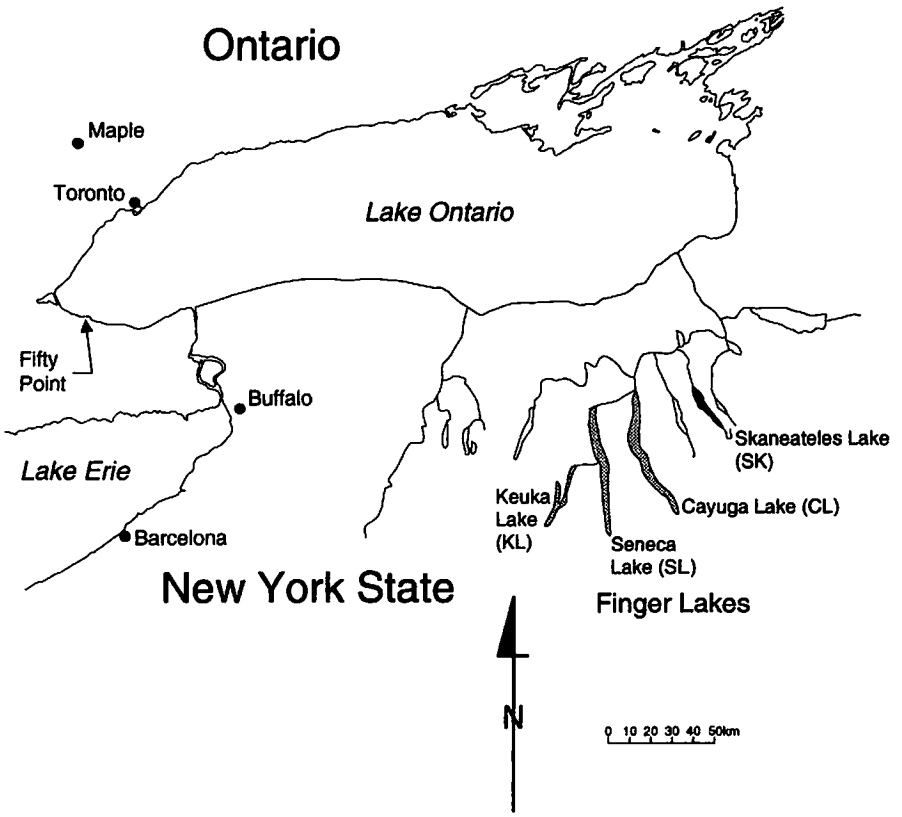

FIGURE 1.—Sources of fish for this study. The Finger Lakes with Atlantic salmon populations are labeled (SL = Seneca, CL = Cayuga, KL = Keuka, and SK = Skaneateles). Stippling represents those Finger Lakes where the Cayuga syndrome has been identified; SK (solid shading) is the only Finger Lake where Atlantic salmon are presently viable.

(all-*rac-alpha*-tocopherol) and C (L-ascorbate) were considered because the cardiovascular lesions, ataxia, and muscular weakness evidenced in moribund sac fry (Fisher et al. 1995b) resembled deficiency signs of these antioxidants observed in other species (Combs 1992). Vitamin A (all-*trans*-retinol) was tested because the abnormal phototaxis seen in syndrome-afflicted sac fry (Fisher et al. 1995b) suggested clinical involvement of the eye and thus a possible vitamin A deficiency (Combs 1992). The multivitamin mixture included the full complex of B vitamins, as well as vitamins D, K, A, C, and E; it was incorporated here to address the potential for the deficiency of a vitamin(s) that was not tested singly.

Vitamin microinjection trial.—Doses of vitamins injected into Atlantic salmon sac fry were based upon requirements (REQ = mg/kg per day) estimated from data determined in older salmonid fishes (National Research Council 1981). Doses were based on an average sac fry wet weight of 206 mg, derived from 200 randomly selected CL sac fry. Water-soluble vitamins were prepared in Cavanaugh's freshwater fish saline (Russell 1990) with 10% dimethylsulfoxide (DMSO) added as a carrier solvent. Vitamins A and E were dissolved in 100% ethanol. Nominal doses of the following vitamins were thus administered: (1) thiamine hydrochloride, 40 μg/g = 8.24 μg/sac fry as thiamine (roughly 200× REQ), (2) L-ascorbate, 100 μg/g = 20.6 μg/sac fry (roughly 200× REQ), (3) all-*rac-alpha*-tocopherol, 100 μg/g = 20.6 μg/sac fry (roughly 100× REQ), (4) all-*trans*-retinol, 225 μg/g = 46.3 μg/sac fry (roughly 10× REQ). The multivitamin inoculum provided the following nominal doses for each sac fry: 975 ng thiamine (roughly 24× REQ), 1.63 IU vitamin A, 0.033 IU cholecalciferol, 0.02 IU vitamin E, 78 μg calcium ascorbate, 1.1 μg riboflavin, 6.5 μg niacine, 6.5 μg pantothenic acid, 1.3 μg pyridoxine hydrochloride, 3.9 ng cyanocobalamin, 65 ng folate, 24 ng biotin, and 8.1 ng vitamin K.

Cayuga Lake Atlantic salmon sac fry injected with vitamins were from progeny of two females (i.e., CL 1-93 and CL 3-93). Little Clear Pond sac fry were also injected, and these were from progeny of 10 females. The LC sac fry served as a toxicity control for each vitamin tested. Sac-fry ages varied between 618 and 676 degree-days (dd), where dd = incubation temperature in degrees centigrade · days postfertilization. Sac fry were anesthetized in 100 mg tricaine methanesulfonate (MS-222)/L and transferred by pipette from the anesthetic bath to a wet, unbleached paper towel under a 4× magnifying lamp. Vitamin solutions were injected into the yolk sac by handheld 50-μL syringe (Hamilton Inc., Reno, Nevada) calibrated to deliver 1 μL per injection via a push-button dispenser. A second 1 μL drop of the water-soluble vitamins (thiamine hydrochloride, L-ascorbate, and the multivitamin mixture) was applied directly onto the gills.

After being injected, the sac fry were incubated in screened, 7.6-cm-diameter polyvinylchloride (PVC) cups in flow-through (1–2 L/min), ambient temperature (8–12°C), dechlorinated tap water that originated from CL (Fisher et al. 1995a). Two replicates of 32 to 40 CL and LC sac fry were monitored for each vitamin tested. Mortality was monitored every 1–3 d until 2 weeks after presentation of food began. At this time, the replicates of thiamine-treated CL fry were pooled and transferred to a 40-L grow-out aquarium to accelerate their growth before release, approximately 1 month later. The LC fry were also pooled at this time but were housed in a separate aquarium for feeding.

Statistical comparisons of survival frequency were evaluated, when appropriate, with chi-square analysis (Zar 1974). Differences in survival were significant if $P < 0.05$.

Thiamine immersion trials.—Atlantic salmon sac fry used for these experiments were from four female parents (CL 3, KL 2, SL 6, and SL 8) representing each of the Finger Lakes stocks affected by the Cayuga syndrome (Fisher et al. 1995a). When thiamine treatment was applied, the sac fry had developed for 675 dd (CL and KL) or 677 dd (SL). Solutions were prepared in filtered, dechlorinated tap water that originated from naturally buffered CL. Relevant chemical variables of CL water have been described (Youngs and Oglesby 1972; Fisher et al. 1995a). Treated sac fry were immersed for 1 h in 1% thiamine hydrochloride (as thiamine) with 0.1% DMSO at pH 5.5. Untreated sac fry were immersed for 1 h in 0.1% DMSO at pH 5.5.

Sac fry from the thiamine immersions were incubated in separate PVC cups as described, but these were housed in a separate 40-L, flow-through (2–3 L/min) aquarium. Mortality was recorded every 1–2 d until 2 weeks after the initial presentation of food. At this time, the treated fry from each female were released into the surrounding 40-L aquarium to accelerate their growth before release about 1 month later.

Stimulus-provoked swimming assay.—To evaluate the effectiveness of thiamine at treating the neurological signs of the Cayuga syndrome (e.g., abnormal phototaxis and thigmotaxis), light-induced swimming behavior was assessed in moribund Atlantic salmon sac fry before and after thiamine treatment. Three sac fry, each from 2 CL and 4 SL females ($N = 18$), were assayed by methodology previously described (Fisher et al. 1995b). Before thiamine treatment, each fish was videotaped individually for 4 min in a lighted, circular, open field. After being videotaped, the sac fry were transferred to individual, perforated plastic test tubes and immersed 1 h in a 1% thiamine hydrochloride bath. After treatment, the tubes containing the sac fry were transferred to a flow-through bath of dechlorinated CL tap water. About 48 h later, the sac fry were videotaped for an additional 4 min, then were returned to the perforated test tubes and incubated for an additional 3 weeks until the completion of yolk absorption necessitated their transfer to a feeding tank. The time spent swimming before and after thiamine treatment was quantified to the nearest second from the video recordings. These data were statistically analyzed by the two-way Mann–Whitney nonparametric test (alpha = 0.05) to compare median swimming time before and after thiamine treatment (Zar 1974). A selection of the recordings was digitized by previous methods (Fisher et al. 1995b) to demonstrate the qualitative differences in swimming behavior in the sac fry after thiamine treatment.

Whole body thiamine analyses.—Live sac fry from individual Atlantic salmon females were frozen in liquid nitrogen, stored at −80°C, and assayed for total thiamine by the thiochrome method (AOAC International 1990). These analyses were performed by Woodson-Tenent Laboratories, Inc. (Memphis, Tennessee). Briefly, the analysis involved: (1) sample homogenization, (2) extraction of sample with 0.1 N HCl, (3) overnight incubation of autoclaved sample with phosphatase to hydrolyze thiamine phosphate esters, (4) ion exchange chromatography to remove cleaved phosphates, (5) elution of total thiamine with potassium chloride, (6) reaction of eluate with alkaline ferricy-

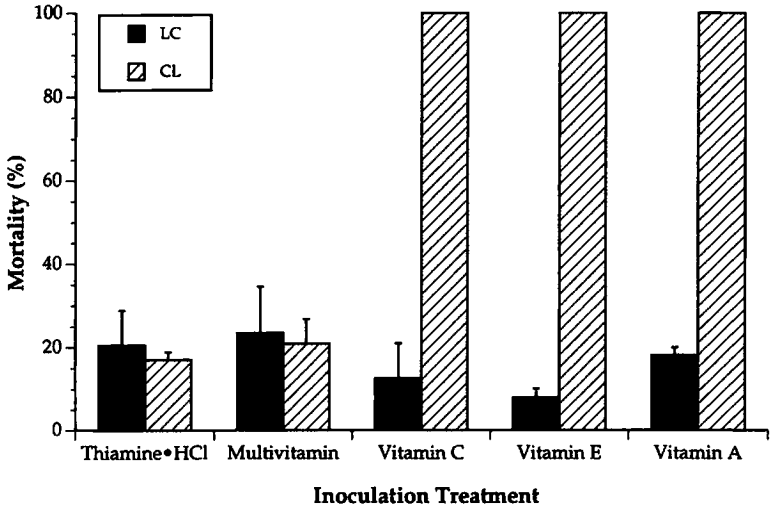

FIGURE 2.—Effect of vitamin inoculation on survival of syndrome-afflicted Atlantic salmon sac fry through the completion of yolk absorption, 2 weeks after the initial presentation of food. Mean percent mortality (+SD) of Cayuga Lake (CL) and Little Clear Pond (LC) sac fry based on two separately cultured replicates of 32–40 sac fry for each vitamin tested.

anide to oxidize thiamine to thiochrome, (7) final extraction with isobutanol, and (8) fluorometric detection of the thiochrome product.

A single 8.5–20.1-g aliquot of whole, homogenized sac fry (roughly 40 to 100 sac fry/sample) was analyzed from each female parent. Spike sample recoveries from 10-g replicate samples of sac fry from a salmonid hybrid (brown trout female *Salmo trutta* × Atlantic salmon male) were 95 and 104%. Sac fry from CL (N = 3) and SL (N = 4) had developed for 665 or 670 dd and exhibited clinical signs typical of the Cayuga syndrome (Fisher et al. 1995b), including convulsive swimming, yolk opacities, and mild subcutaneous edema. Control LC sac fry (N = 6) were slightly older (725 dd) because of an earlier spawning date. Replicates from each female were not analyzed because of limitations in the number of sac fry required for a single analysis. Likewise, progeny from several females were not analyzed because of limitations in the number of sac fry available from these parents on the date the samples were taken.

Unfertilized lake trout eggs from the control and experimental stocks were frozen at −20°C before thiamine analysis and analyzed by the thiochrome method (AOAC International 1990) by Hazelton Laboratories (Madison, Wisconsin). A single 10-g aliquot of unfertilized eggs (roughly 100 eggs) was analyzed from each female. Product recovery was not assessed in the lake trout samples. Survival of the lake trout fry from each female was regressed against thiamine concentration in the eggs. The slope (β) of this regression was evaluated for significance against the null hypothesis of $\beta = 0$ (Zar 1974).

Results

Vitamin Injection Therapy

Thiamine treatment by yolk-sac injection eliminated the mortality associated with the Cayuga syndrome (Figure 2). There was no significant difference in mortality between the CL sac fry injected with the multivitamin and those injected with thiamine hydrochloride. There was also no significant difference between the survival of the negative-control LC progenitor stock and the CL sac fry injected with either thiamine or the multivitamin. There was no additional mortality observed in the thiamine-treated CL sac fry after they were pooled, and feeding was normal as evidenced by their rapid growth during the final month before release.

The CL sac fry inoculated with vitamins C, E, and A all eventually succumbed to the Cayuga syndrome (Figure 2). Death occurred between 700 and 950 dd, consistent with the uninjected positive-control animals of previous and simultaneous studies (Fisher et al. 1995a). The mortality of the negative-control LC sac fry (average, all treatments = 16.6, SE = 3.17) was consistent with injection trauma (Black et al. 1985).

TABLE 1.—Effect of a 1-h, 1% active thiamine bath on total mortality (%) of Atlantic salmon sac fry with Cayuga syndrome, and degree-days to 75% mortality for an equal number of untreated sac fry from the same female parent.

Statistic	Source of sac fry[a]			
	CL 3	SL 6	SL 8	KL 2
Number of treated sac fry[b]	62	75	100	23
Total mortality of treated[c] sac fry	3.28	1.33	4.0	0.0
Total mortality of untreated sac fry	100	100	100	100
Degree-days[d] to 75% mortality of untreated sac fry	814	804	804	738

[a] Lake source and parental identification number of sac fry immersed in a thiamine bath (CL = Cayuga Lake, SL = Seneca Lake, KL = Keuka Lake). All female parents were spawned in November 1993.

[b] The number includes the number of sac fry treated with a thiamine bath and also the number untreated; it represents all the sac fry that remained alive from each female at the time of treatment.

[c] Mortality was monitored for 2 weeks after initial presentation of food.

[d] The number of days postfertilization multiplied by the incubation temperature (°C).

Thiamine Bath Treatments

A single 1-h aqueous exposure to 1% active thiamine proved sufficient to eliminate syndrome-related mortality, regardless of the female or lake source of afflicted sac fry (Table 1). No mortality was recorded in the negative-control LC sac fry exposed to the same treatments over the same 6-week period. These results corroborated preliminary trials in which moribund Atlantic salmon sac fry from CL and SL were immersed 1 h in 1% thiamine every 4–6 d for 1 month (unpublished data).

Behavioral Responses to Thiamine Treatment

The abnormal thigmotaxis, phototaxis, and convulsive swimming usually observed earlier in the progression of the Cayuga syndrome (Fisher et al. 1995b) was seen in only 5 of the 18 fish assayed before thiamine treatment (Figure 3). The remainder of the syndrome-afflicted sac fry assayed did not swim and were unresponsive to the aversive light stimulus, a stage usually seen 1–4 d before death. Four of the sac fry tested died before the posttreatment assay was performed 48 h later.

After thiamine treatment, the stimulus-provoked sac fry swam along the edge of the circular observation vessel until fatigued (Figure 3). This 'edging' behavior was characteristic of the positive thigmotaxis and negative phototaxis exhibited by the normal LC sac fry at this developmental stage (Fisher et al. 1993; Fisher et al. 1995b). The average time spent swimming increased over 40-fold after thiamine treatment ($\text{mean}_{\text{before}}$ = 3.2 s, SE = 1.3 s; $\text{mean}_{\text{after}}$ = 107 s, SE = 18.6 s), a highly significant finding ($U = 15$, $P \leq 0.0001$) consistent with neurological recovery (Figure 4). Each of the 14 sac fry assayed after thiamine treatment survived until the initiation of feeding, when the experiment was concluded.

Relation of Thiamine Level to the Cayuga and Swim-up Syndromes

Evidence supporting a dietary link to the Cayuga syndrome was provided by analyses of whole body thiamine from moribund Atlantic salmon sac fry (Figure 5). Thiamine concentrations were at or near the 100 ng/g detection limit of the thiochrome method in both the CL and SL salmon. In contrast, total thiamine in the negative-control LC sac fry

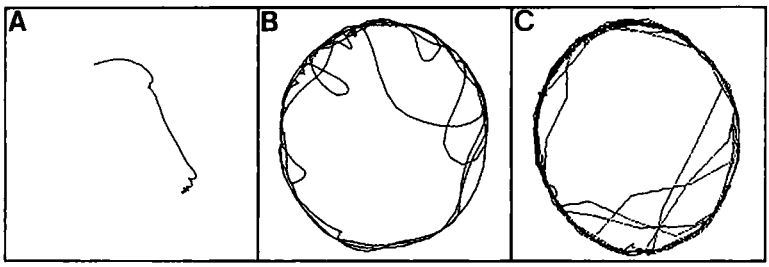

FIGURE 3.—Digitized swimming patterns of single Atlantic salmon sac fry from the control Little Clear Pond (LC) stock and from the syndrome-afflicted Cayuga Lake (CL) stock before and after thiamine bath treatment. Drawings indicate (**A**) representative syndrome-afflicted CL Atlantic salmon sac fry before thiamine bath treatment; (**B**) swimming pattern of the same CL sac fry 48 h after thiamine bath treatment; and (**C**) representative normal swimming pattern of LC sac fry from the 1992–1993 season (see Fisher et al. 1995b). Digitized patterns of the syndrome-afflicted CL sac fry before and after thiamine treatment were graciously provided by Aaron DeLonay, National Biological Survey, Columbia, Missouri.

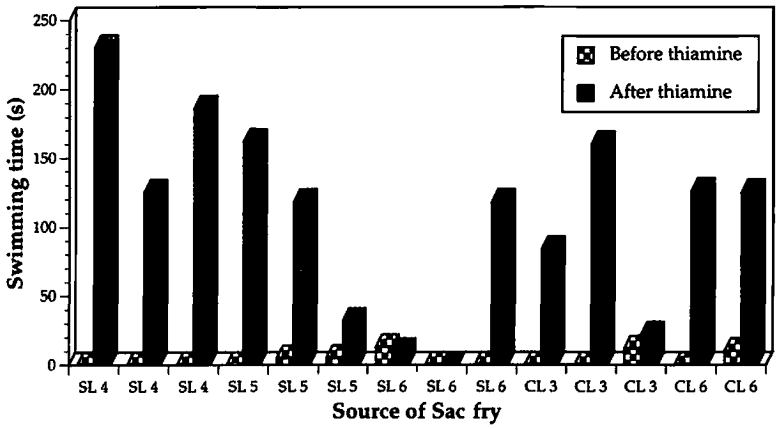

FIGURE 4.—Effect of a 1-h, 1% thiamine bath on the light-induced swimming behavior of Atlantic salmon sac fry afflicted with the Cayuga syndrome. Each three-dimensional bar represents the swimming time of one syndrome-afflicted sac fry during a 4-min observation before and 48 h after thiamine treatment. The source of the sac fry is indicated by the female parent identification number from the autumn 1993 broodstock collections and by the lake source (Cayuga Lake = CL; Seneca Lake = SL).

averaged 615 ng/g (SE = 64.9). The relatively low concentration of thiamine detected in negative-control sac fry from LC 7 (234 μg/g wet weight) had no apparent effect on survival (100%). Survival of all progeny from the LC salmon was 98 to 100% from hatching (450–500 dd) until the initial presentation of food at the completion of yolk absorption (950 dd). Given the extreme difference in the thiamine levels between the control and syndrome-afflicted Atlantic salmon, further statistical analysis was not considered.

Thiamine analyses of the three lake trout stocks evaluated in this study also suggest a dietary link to the swim-up syndrome of this species. Notably,

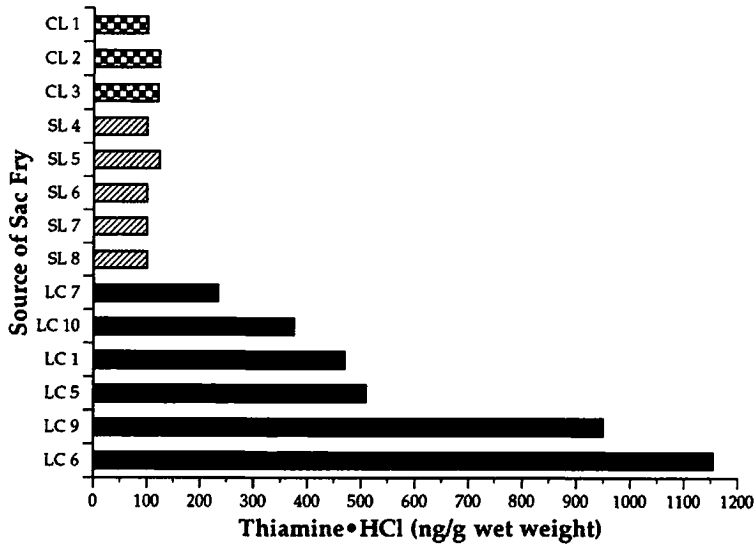

FIGURE 5.—Whole body thiamine concentrations of syndrome-afflicted and control Atlantic salmon sac fry represented in this study (1993–1994 season). The female number and lake source of the sac fry are indicated; Little Clear Pond (LC) is the negative-control progenitor stock; salmon from Cayuga Lake (CL) and Seneca Lake (SL) are afflicted with Cayuga syndrome. Each bar represents the result of a single analysis of pooled, homogenized sac fry (8.5–20.1 g) from a single female parent, except the bar for CL 1, which represents the average of two samples (no variation).

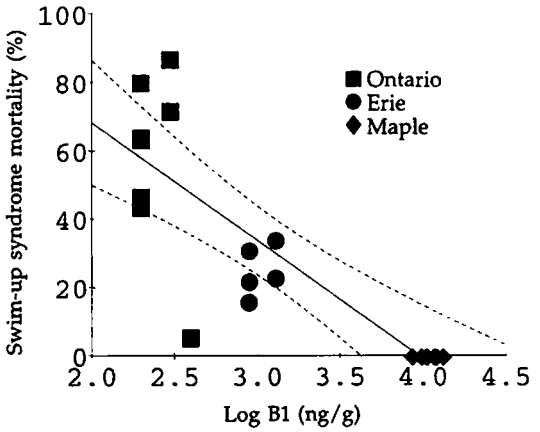

FIGURE 6.—Regression of $\log_{10}$ thiamine (B1) content of lake trout eggs and swim-up syndrome mortality. Each datum represents a single analysis of approximately 10 g of eggs from a single female. Maple lake trout eggs represent the hatchery control stock whose parents (N = 7) were fed a thiamine-supplemented diet; eggs from Lake Ontario were derived from broodstock captured near Stony Island (N = 6) and Fifty Point (N = 2); eggs from Lake Erie were derived from broodstock captured in Barcelona Harbor (N = 5). Dashed lines are 95% confidence intervals.

there was a significant inverse relation ($P < 0.001$, $r^2 = 0.72$) between the $\log_{10}$ concentration of thiamine hydrochloride in lake trout eggs and the mortality of the resultant fry that exhibited clinical signs of the swim-up syndrome (Figure 6). Mortality of lake trout that hatched successfully but later displayed the swim-up syndrome was 57.9% (SE = 9.14) for the Lake Ontario fry and 25.2% (SE = 3.24) for the Lake Erie fry.

Discussion

The diets of salmonids in the Finger Lakes and Lakes Ontario and Erie include native percids, coregonids, cottids, and invertebrates, as well as the nonnative rainbow smelt and alewife (Youngs and Oglesby 1972; Christie 1974; Elrod 1983; Brandt 1986; Hartman 1988). The associations of the Cayuga and swim-up syndromes to a diet of alewives are consistent with previous studies of Chastek's Paralysis, in which captive foxes *Vulpes* sp. (Green and Evans 1940; Okada et al. 1987), chicks *Gallus domestica* (Spitzer et al. 1941), domestic cats *Felis catus* (Smith and Proutt 1944), mink *Mustela vison* (Gnaedinger 1964; Okada et al. 1987), and fish *Schilbeodes* (=*Noturus*) *mollis* and banded sunfish *Enneacanthus obesus* (Harrington 1954) were fed experimental or production diets of thiaminase-rich fish or fish products. Although thiaminase has been detected in rainbow smelt (Deutsch and Ott 1942; Nielands 1947; Gnaedinger 1964) and several coregonids (Deutsch and Hasler 1943; Nielands 1947; Gnaedinger 1964), far greater activity has been found in alewives (Gnaedinger 1964). Furthermore, rainbow smelt and coregonids are considered secondary to the alewife as prey (Youngs and Oglesby 1972; Elrod 1983; Brandt 1986).

Forage is less diverse in Lake Ontario than in Lake Erie because of the virtual elimination of native prey fishes by competition from alewives (Smith 1970). Thus, the difference in thiamine concentrations between the Lake Ontario and Lake Erie lake trout eggs may reflect the greater abundance of the thiaminase-rich alewife in the diet of Lake Ontario lake trout. Of note, early mortality syndromes have not been reported from Lakes Huron or Superior, where alewives represent only a minor proportion of the salmonid diet (Dryer et al. 1965; Diana 1990) because of their low abundance (Smith 1970; Bronte et al. 1991).

The severe thiamine deficiency in landlocked Atlantic salmon in the Finger Lakes may account for their death earlier in development than that seen in lake trout from Lakes Ontario and Erie. Alternatively, Atlantic salmon may be more sensitive than lake trout to thiaminase. However, the thiamine status of these species was not assessed at similar developmental stages. A continual decline in thiamine content was observed in rainbow trout from fertilization to first feeding (Sato et al. 1987); thus, the Atlantic salmon may have begun embryogenesis with thiamine levels similar to those of the lake trout. Such a scenario would imply a greater requirement for thiamine in developing Atlantic salmon than in the lake trout. The present results imply that the whole body thiamine requirement of Atlantic salmon to complete yolk absorption and feed successfully lies somewhere between 124 μg/g, the highest amount detected in syndrome-afflicted sac fry, and 234 μg/g, the lowest level detected in the LC sac fry.

The extreme thiamine deficiency of the Finger Lakes Atlantic salmon more plausibly reflects the epilimnetic foraging behavior of this species (Lackey 1970; Speirs 1974). Like the Atlantic salmon, alewives prefer the warmer waters above the thermocline (Brandt et al. 1980) and are largely epilimnetic during summer and fall (Lackey 1970; Jude et al. 1987). We propose that for the 5–6 months before Finger Lakes Atlantic salmon spawn, the fish feed nearly exclusively on thiaminase-rich alewives and thereby compromise the transovarian deposition of thiamine. Lake trout and brown trout in the

Finger Lakes do not exhibit a similar reproductive problem, probably because these species prefer the colder waters within and below the thermocline and thereby encounter a more diverse diet during the 3 months (lake trout) to 6 months (brown trout) preceding their autumnal spawning (Jude et al. 1987). This schema was suggested to explain the reduction of alewives and increase in smelt observed in the stomachs of Lake Ontario lake trout during the summer (Elrod 1983). The higher survival of the Lake Ontario and Lake Erie lake trout relative to the Finger Lakes Atlantic salmon would also be supported by such a dietary shift.

These results have shown that the mortality and behavioral impairment associated with the Cayuga syndrome of Finger Lakes Atlantic salmon can be alleviated with thiamine treatment. To our knowledge, this thiamine-responsive Cayuga syndrome represents the first case of a vitamin deficiency known to cause the complete reproductive failure of an animal population from a natural environment. Results of thiamine analyses of lake trout stocks with the swim-up syndrome further support the necessity of thiamine for the survival of lake trout fry (Fitzsimons 1995).

The present findings suggest that a thiamine deficiency may be involved in similar mortality syndromes of other populations of Great Lakes salmonids. Indeed, preliminary thiamine treatment experiments with Lake Michigan steelhead trout *O. mykiss* reduced swim-up mortality from 38 to 23.8% (M. W. Hornung, University of Wisconsin, personal communication). The role of thiamine in the catastrophic M-74 syndrome of larval Atlantic salmon (Norgrenn et al. 1993) from the Baltic Sea is also under consideration. This syndrome afflicts the sac fry progeny of the anadromous Baltic salmon with a mortality pattern and pathological signs similar to those of the Cayuga syndrome. Notably, adult Baltic Atlantic salmon feed heavily on thiaminase-rich clupeids (Nielands 1947). Indeed, preliminary results with the methods discussed herein indicate that thiamine may be effective at reducing M-74 mortality also (Bylund and Lerche 1995).

With the previous invasions of the alewife (Miller 1957) and sea lamprey (Lawrie 1970), the intentional introduction of the Pacific salmonids *Oncorhynchus* spp. (Christie 1974) and the recent ballast-water entries of the zebra mussel *Dreissena* sp. (Griffiths et al. 1991), ruffe *Gymnocephalus cernuus* (Pratt et al. 1992), and at least two species of freshwater goby (Jude et al. 1992), the Great Lakes basin represents the most taxonomically disturbed, temperate freshwater ecosystem (Mills et al. 1993). The competitive and predatory effects of these nonnative species on native fauna have been substantial (Smith 1970; Christie 1974; Hartman 1988). The present epizootiological evidence connecting reproductive problems of two native species to a diet of nonnative alewives suggests an additional nutritional mechanism by which trophic balance can be disrupted. We propose that the failure of some stocking programs to establish self-sustaining salmonid populations in Lakes Ontario, Erie, and Michigan is, in part, the result of the antagonistic effect of alewife forage on thiamine nutrition.

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