

Interspecies Comparisons of Blood Thiamine in Salmonids from the Finger Lakes, and Effect of Maternal Size on Blood and Egg Thiamine in Atlantic Salmon with and without Cayuga Syndrome

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Abstract.—A lethal thiamine deficiency afflicting larval landlocked Atlantic salmon *Salmo salar* in several of New York's Finger Lakes has been linked to a maternal diet of the exotic, thiaminase-rich alewife *Alosa pseudoharengus*. To evaluate why trout and char species in the Finger Lakes are apparently not affected by this "Cayuga syndrome," levels of thiamine in the whole blood of syndrome-positive and syndrome-negative stocks of Atlantic salmon were compared with levels in lake trout *Salvelinus namaycush*, brown trout *Salmo trutta*, and rainbow trout *Oncorhynchus mykiss* from Cayuga and/or Seneca lakes. Thiamine levels did not differ between sexes within any species or stock. Consistent with the hypothesis that thermal habitat partitioning may predispose the salmon to more dietary thiaminase than other Finger Lakes salmonids, thiamine levels in the salmon that produced syndrome-positive sac fry were significantly lower than levels measured in Finger Lakes brown trout and rainbow trout. In contrast, there was no difference between the syndrome-positive salmon and Finger Lakes lake trout, possibly because the male char were in starved (postspawned) condition. Regressions of maternal blood or egg thiamine versus maternal weight and length were not significant for salmon that produced syndrome-positive sac fry; yet, a significant inverse relationship was detected for the syndrome-negative salmon from the Adirondack progenitor stock. These findings may reflect the transition of these reference control salmon from a thiaminase-poor invertebrate diet to a piscivorous diet of thiaminase-active smelt *Osmerus mordax*.

Reproduction is impaired in salmonid populations that grow to maturity in the Finger Lakes of New York State (Fisher et al. 1995a), the lower Great Lakes of Canada and the United States (Mac et al. 1985; Skea et al. 1985; Mac and Edsall 1991; Fitzsimons et al. 1995), and the Baltic Sea (Norrgrén et al. 1993). In each of these regions, reproductive impairment is observed as "early mortality syndromes" in sac fry (i.e., alevins) or

first-feeding fry. In the Finger Lakes (FL), the Cayuga syndrome has afflicted sac fry of landlocked Atlantic salmon *Salmo salar* since at least 1974, when it was first observed (Fisher and Spitsbergen 1990; Fisher et al. 1995a). Affected salmon populations previously identified include those in Cayuga Lake (CL), Seneca Lake (SL), and Keuka Lake (KL). Recent studies have also documented the condition in Atlantic salmon from Green Pond (GP) in the Adirondack Mountains and Otsego Lake (OL), the headwaters of the Susquehanna River (Fisher et al. 1996a).

Every adult female Atlantic salmon captured from CL, SL, and KL produced sac fry with the Cayuga syndrome, and mortality in these sac fry nearly

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always reached 100% (Fisher et al. 1995a). Nutrition was implicated as a possible mediator of the disease because only populations with diets that included the nonnative, thiaminase-rich alewife *Alosa pseudoharengus* produced sac fry that exhibited syndrome-related mortality (Fisher and Spitsbergen 1990; Fisher et al. 1995a). Evaluations of gross lesions and neurological signs in sac fry with Cayuga syndrome (Fisher et al. 1995b), reduction of mortality after thiamine treatment in lake trout *Salvelinus namaycush* swim-up fry (Fitzsimons 1995) and syndrome-positive Atlantic salmon sac fry (Fisher et al. 1996b), and depressed thiamine concentrations in syndrome-positive offspring of both species (Fisher et al. 1996b) gave strong support to the role of thiamine in these early mortality syndromes.

Multiple salmonid species suffer syndrome-related mortalities in the Great Lakes (Marcquenski and Brown 1997) and Baltic Sea (Norrgrén et al. 1998, this volume). In contrast, Cayuga syndrome has been observed in landlocked Atlantic salmon only within the affected FL (Fisher et al. 1995a). For example, the New York State Department of Environmental Conservation (NYSDEC) routinely spawns lake trout from SL for stocking programs and has not previously documented the early mortality syndromes seen in Great Lakes lake trout (Fitzsimons et al. 1995). Likewise, viability investigations of CL rainbow trout *Oncorhynchus mykiss* (Skea et al. 1985) and brown trout *Salmo trutta* (Fisher 1995; Fisher et al. 1995a) did not reveal syndrome-related mortality.

The apparent sensitivity of FL Atlantic salmon to Cayuga syndrome has been a source of confusion and contention. Fisher et al. (1996b) suggested that the Atlantic salmon consumed more thiaminase-rich alewife than other salmonid species because of the preference of both the salmon and the alewife for epilimnetic waters before the salmon's fall spawning season (Lackey 1969, 1970; Brandt et al. 1980; Haynes 1995). Under this hypothesis, the salmon would be exposed to higher concentrations of dietary thiaminase than the more metalimnetic and hypolimnetic brown trout and lake trout (Jude et al. 1987; Haynes 1995). Consequently, the transovarian deposition of thiamine in the salmon would be relatively reduced.

A valid criticism of the "alewife connection" to the Cayuga syndrome is that it ignores other means of inducing thiamine deficiency. For example, the exotic rainbow smelt *Osmerus mordax* is also abundant in the FL and also exhibits thiaminase activity

(Deutsch and Ott 1942; Gnaedinger 1964; Ji and Adelman 1998, this volume). Fisher et al. (1995a) considered it unlikely that smelt were responsible for the thiamine deficiency in FL salmon because smelt were the principal diet of salmon in Little Clear Pond (LC), the FL progenitor stock, and no syndrome-related mortality had been seen in the LC sac fry. Thiaminase activity in smelt was also reported to be roughly one-third that in alewife (Gnaedinger 1964). Furthermore, in waters where smelt and alewife coexisted, smelt were confined to the colder, hypolimnetic and metalimnetic waters (Brandt et al. 1980; Jude et al. 1987; Haynes 1995). Thus, there would be fewer opportunities for FL salmon to consume smelt during the summer months. Finally, lake trout and brown trout feed on smelt to at least some extent during the spring, summer, and fall, yet no syndrome-like reproductive problems have been recorded in FL populations of these species.

To address questions raised by the hypothesized dietary association of Cayuga syndrome, we compared blood levels of each thiamine moiety between FL trout and char stocks and landlocked Atlantic salmon that were or were not thiamine-deficient (i.e., syndrome-positive, SYN[+], or syndrome-negative, SYN[-]). These data provide the first evaluation of thiamine blood profiles from multiple salmonid species within the same systems. Because predator size also influences prey preference (Brandt 1986; Jude et al. 1987), we also sought to establish how thiamine levels in the blood and eggs of the landlocked salmon varied with maternal size and whether a difference existed between populations that produced SYN[+] sac fry and those that did not. This latter question was especially important with respect to earlier investigations of the LC sac fry, whose thiamine levels, although severalfold higher than those of SYN[+] sac fry, were comparable with those of lower Great Lakes stocks of lake trout that exhibited variable levels of swim-up mortality (Fisher et al. 1996b).

Materials and Methods

Source of Fish

All Atlantic salmon sampled were captured by electroshocking tributaries or setting trap nets during the fall 1994 spawning migrations or egg harvests (Table 1). The reference control salmon stock for these studies (i.e., LC) is the progenitor source for all lakes stocked with landlocked salmon in New

TABLE 1.—Source and sex of salmon and trout sampled for these studies.

Species sampled	Source of salmon and trout ^a	Male (N)	Female (N)	UK ^b (N)	Date bled
Atlantic salmon	Little Clear Pond	2	10	0	9 November 1994
<i>Salmo salar</i>	Little Clear Pond Hatchery	0	5	0	9 November 1994
	Green Pond	0	1	0	9 November 1994
	Cayuga Lake	3	3	0	21 November 1994
	Otsego Lake	7	11	0	16 November 1994
	Seneca Lake	2 ^c	0	0	14 and 17 November 1994
Lake trout	Cayuga Lake	1 ^d	0	0	22 May 1994
<i>Salvelinus namaycush</i>	Seneca Lake	4	0	0	17 November 1994
Brown trout	Cayuga Lake	1 ^d	0	0	21 May 1994
<i>Salmo trutta</i>	Cayuga Lake	2	5	4	14 and 15 November 1994
	Seneca Lake	2	0	0	17 November 1994
Rainbow trout	Seneca Lake	3	6	4	14 November 1994
<i>Oncorhynchus mykiss</i>	Seneca Lake	0	0	2	15 November 1994
	Seneca Lake	1	4	2	17 November 1994

^a Salmon, brown trout, and lake trout were sexually mature (i.e., expressed either eggs or milt) at time of sampling, unless otherwise noted.

^b Sex not recorded.

^c Immature yearling salmon, no milt expressed.

^d Sampled from angler catches during annual spring fishing derby; no milt expressed.

York State; this stock subsists naturally on a diet of invertebrates and smelt (Fisher et al. 1995a). The four experimental stocks came from systems with alewife forage (i.e., CL, SL, OL, and GP), and salmon from these systems produce sac fry that die from the Cayuga syndrome (Fisher et al. 1995a, 1996a). Alewives were introduced unintentionally into CL and SL through the building of the Erie Canal in the late 1800s. Alewives were introduced into OL without permission during the late 1980s. Alewives were introduced intentionally into GP in 1957 and 1959 to provide forage for splake *Salvelinus fontinalis* × *S. namaycush*. Three of these systems also have smelt populations (CL, SL, and OL). The hatchery stock (LCH) also originated from LC brood and were fed New York Diet #4, a broodstock diet that is fortified with 2,863 mg/kg thiamine mononitrate. Because smelt also express thiaminase (Gnaedinger 1964; Ji and Adelman 1998), the LCH stock provided a dietary control for this study. Additional stock and watershed characteristics are described elsewhere (Oglesby 1978; Fisher et al. 1995a).

All salmon were ripe at the time of blood sampling except those from CL, one female each from OL and LCH that had already spawned, and the two salmon from SL, which were immature male yearlings. Salmon from CL were transported to the Resource Ecology and Management Facility at Cornell

University, where they were held for 2 weeks until ripe in a 2.6-m (diameter) tank with flow-through, dechlorinated CL water. Salmon from CL and GP were killed immediately before sampling with a blow to the head. Salmon from LC, LCH, and OL were briefly anesthetized in tricaine methanesulfonate (MS-222, Sigma Chemical Co., St. Louis, Missouri) before sampling on site and released.

Trout and char (i.e., rainbow trout, brown trout, and lake trout) were collected during efforts to capture salmon on Cayuga and Seneca lakes, with the exception of one brown trout and one lake trout that were donated (live) by anglers during a spring 1994 fishing derby on CL (Table 1). All brown trout caught in the fall were ripe or nearly ripe. Seneca Lake lake trout spawn from late September to mid-October; hence, although the four males captured from SL still expressed milt, they were gaunt and in poor condition, having spawned probably a month or more earlier. Rainbow trout from SL were captured near the mouth of Katherine Creek at the beginning of their spawning run. Spawning of SL rainbow trout occurs in early March in the headwaters of Katherine Creek; thus, they were not ripe at the time of sampling. All trout were sampled in the field and released or transported back to Cornell University and held for a maximum of 4 d in a tank identical to that used to ripen CL salmon.

Blood and Egg Collection

Blood samples were taken from the caudal vein of all salmon, trout, and char captured. Blood was drawn using 22-gauge needles and Vacutainer™ collection ampules, and samples were frozen directly on dry ice. Twenty eggs from each landlocked Atlantic salmon spawned were also frozen to determine thiamine status. Survival of the sac fry progeny from each of the salmon spawned was evaluated in a related study (Fisher et al. 1996a); these survival results were used in the present report only to identify blood samples from salmon that produced SYN[+] offspring. Survival of the progeny from the FL trout and char was not examined because previous studies have indicated that they were not affected by the Cayuga syndrome (Skea et al. 1985; Fisher 1995) and because limited laboratory facilities precluded the monitoring of offspring survival in all the salmonid species captured.

Thiamine Analyses

Gradient reversed phase high-performance liquid chromatography was used to detect and quantify free thiamine, thiamine monophosphate (TMP), and thiamine pyrophosphate (TPP) in the whole blood of all salmonids sampled and in the egg samples of all salmon spawned. The method is described in detail by Brown et al. (1998, this volume). Product recovery in samples spiked with free thiamine ranged from 85 to 90%; recovery in samples spiked with TPP ranged from 80 to 90%. Assay detection limits were 0.002, 0.004, and 0.005 nmol/g for free thiamine, TPP, and TMP, respectively. The coefficient of variation for an egg sample analyzed 6 times was 7.47%; the coefficient of variation for a blood sample analyzed 10 times was 5.96%.

Statistical Analyses

Blood thiamine comparisons.—Differences in mean levels of thiamine in the whole blood were evaluated for significance using one-way analysis of variance (ANOVA) techniques (Zar 1974). Thiamine levels were log-transformed to adjust for heterogeneous variance. Mean levels of thiamine were compared among (1) stock, species, and sex, and (2) sexes within stocks. Findings of significance ($\alpha \leq 0.05$) were followed with the Bonferroni multiple-comparison test.

If the Bonferroni multiple-comparison test did not reveal differences between stocks or between sexes within stocks, then stocks were pooled by spe-

cies (e.g., blood data from male and female CL and SL brown trout were pooled into "FL brown trout"). The pooling of blood data from salmon stocks was modified slightly from that of trout and char stocks in that it was specifically performed to examine differences between SYN[+] and SYN[-] salmon. For female salmon, syndrome status was determined based on the survival of their sac fry progeny, as discussed above (Fisher et al. 1996a). Because no correlate of thiamine status to male salmon viability has been specifically identified, the syndrome status of male salmon was assigned according to whether their blood levels differed significantly from those of the females of the same stock, unless maturity levels of the salmon would have prevented their consideration as SYN[+]. Thus, the SYN[-] Atlantic salmon group ($N = 17$) consisted of all male and female salmon from LC, the three female salmon from OL whose progeny did not die from the Cayuga syndrome (Fisher et al. 1996a), and the two immature SL salmon; the LCH salmon were excluded from the SYN[-] group because of their artificial, thiamine-fortified diet. The SYN[+] group ($N = 22$) consisted of all salmon from CL, GP, and OL, except for the three OL females just described.

Thiamine levels versus maternal size.—Regression analyses were performed to determine whether maternal length or weight of landlocked Atlantic salmon related to the thiamine levels in their blood or eggs. Initially, data from all female salmon were pooled, and regression analyses were performed for each of the thiamine moieties. These graphic analyses suggested clustering of the data that closely corresponded to the SYN[-] and SYN[+] groups (i.e., including both sexes) described in the preceding section. Subsequent regressions were performed to examine whether the effect of maternal size on blood and egg thiamine was different among (1) reference control LC salmon, (2) SYN[-] salmon, and (3) salmon that produced SYN[+] sac fry ($H_0, \beta = 0$; $H_a, \beta \neq 0$). The SYN[-] group included the control LC females, and the three OL females that produced normal sac fry (Fisher et al. 1996a). The SYN[+] group included the three female CL salmon and the seven female OL salmon whose sac fry died from Cayuga syndrome in the associated study (Fisher et al. 1996a). No weight or length data were available for the GP (SYN[+]) female. Likewise, the LCH salmon were excluded from the SYN[-] group because of their artificial diet. All statistical calculations were assisted by Data Desk version 4.1 (Data Description, Inc., Ithaca, New York).

Results

Interspecies and Stock Comparisons of Blood Thiamine

The principal form of thiamine detected in the whole blood of all species and stocks was the pyrophosphate moiety, constituting from 70.7 (SL rainbow trout) to 90% (SL brown trout) of the total thiamine in the systemic circulation (Figure 1). Concentrations of TMP and free thiamine were more variable between stocks and species than TPP or total thiamine levels (Figure 2). Thiamine monophosphate ranged from 3.5 to 20% of total thiamine in the blood, whereas free thiamine ranged from 2.9 to 14.9% of the total.

Thiamine levels did not differ between sexes within stocks, so data from both sexes were pooled by stock and ANOVAs were computed again. Levels of each thiamine moiety differed between stocks and species ($F_{\log \text{TMP}} = 9.065, df = 11, 78; F_{\log \text{TPP}} = 8.365; F_{\log \text{free thiamine}} = 8.0783; F_{\log \text{total thiamine}} = 11.426$). Reflective of their diet fortified with free thiamine, the LCH stock had the highest levels of systemic thiamine for nearly every moiety (Figures 1 and 2); however, the levels did not differ significantly from those found in the SYN[-] LC salmon. Thiamine levels in the blood of the naturally fortified SL rainbow trout were also exceptionally high and did not differ from those of the LC control stock. Despite

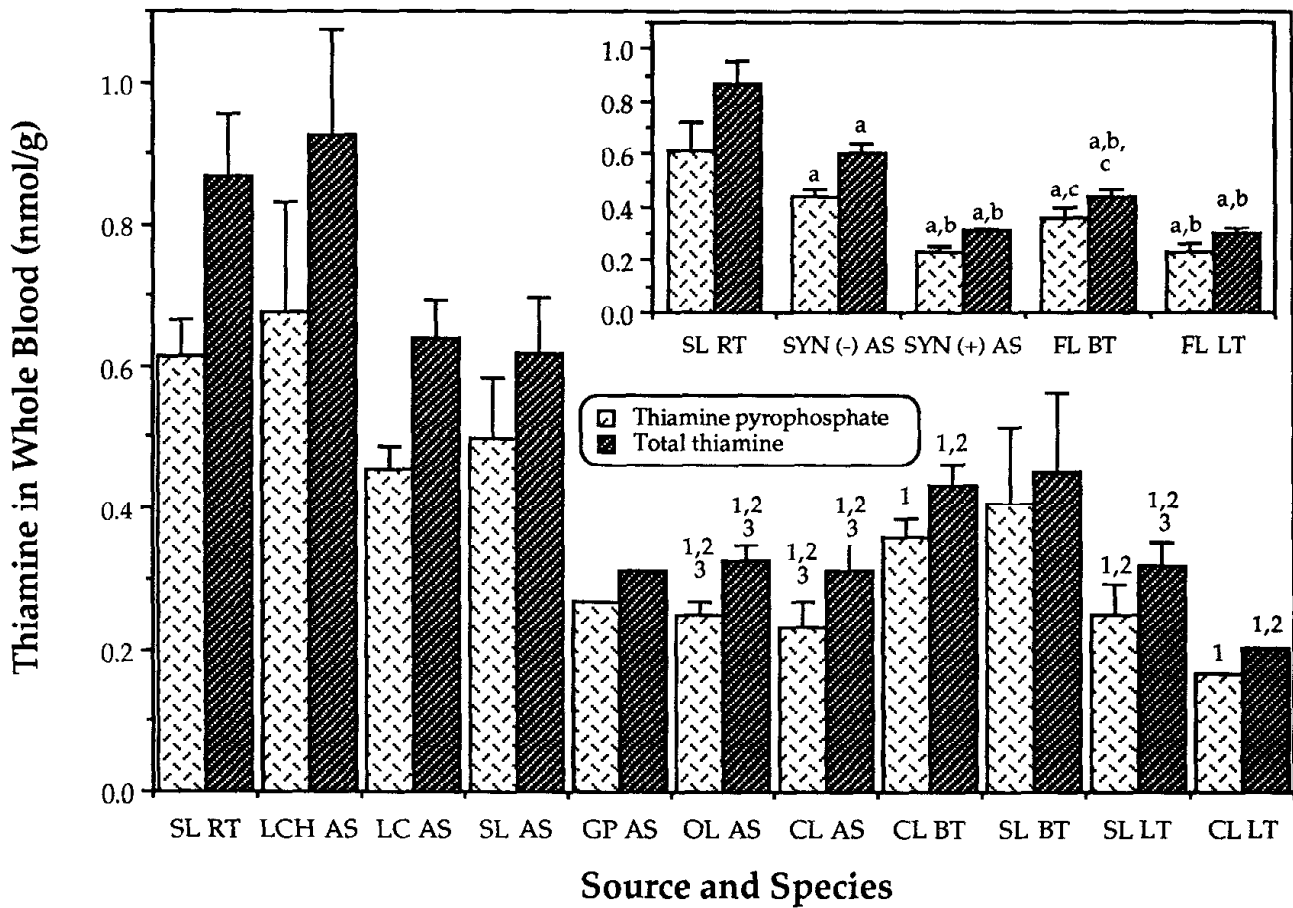


FIGURE 1.—Thiamine pyrophosphate and total thiamine concentrations in the whole blood of salmon, trout, and char populations from lakes with syndrome-positive (SYN[+]) and syndrome-negative (SYN[-]) Atlantic salmon stocks. Codes, from left to right, depict the following stocks and species: Seneca Lake rainbow trout (SL RT, $N = 22$), Little Clear Hatchery Atlantic salmon (LCH AS, $N = 5$), Little Clear Pond Atlantic salmon (LC AS, $N = 12$), Seneca Lake Atlantic salmon (SL AS, $N = 2$), Green Pond Atlantic salmon (GP AS, $N = 1$), Otsego Lake Atlantic salmon (OL AS, $N = 18$), Cayuga Lake Atlantic salmon (CL AS, $N = 6$), Cayuga Lake brown trout (CL BT, $N = 17$), Seneca Lake brown trout (SL BT, $N = 2$), Seneca Lake lake trout (SL LT, $N = 4$), and Cayuga Lake lake trout (CL LT, $N = 1$). Significance of multiple comparisons are depicted as: 1 = different from SL RT, 2 = different from LCH AS, and 3 = different from LC AS ($P \leq 0.05$). The inset depicts the pooled species or stock data as described in the text. Categories from left to right are: SL RT ($N = 22$), SYN[-] Atlantic salmon ($N = 17$); SYN[+] Atlantic salmon ($N = 22$), Finger Lakes (FL) brown trout ($N = 5$), and FL lake trout ($N = 4$). Significance of multiple comparisons depicted in the inset are as follows: a = different from SL RT, b = different from SYN[-] AS, and c = different from SYN[+] AS ($P < 0.05$).

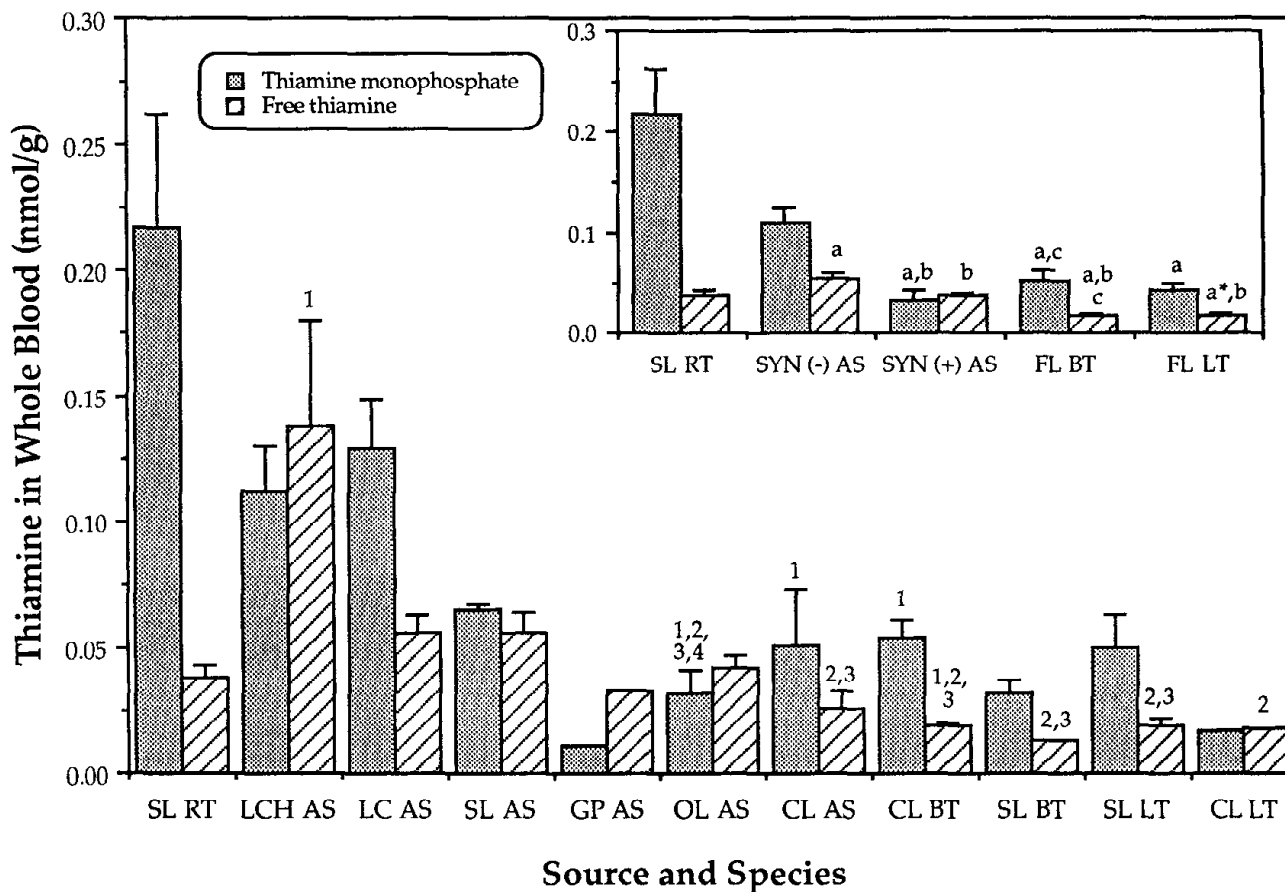


FIGURE 2.—Thiamine monophosphate and free thiamine concentrations in the whole blood of salmon, trout, and char populations from lakes with syndrome-positive (SYN[+]) and syndrome-negative (SYN[−]) Atlantic salmon stocks. Codes, from left to right, depict the following stocks and species: Seneca Lake rainbow trout (SL RT, $N = 22$), Little Clear Hatchery Atlantic salmon (LCH AS, $N = 5$), Little Clear Pond Atlantic salmon (LC AS, $N = 12$), Seneca Lake Atlantic salmon (SL AS, $N = 2$), Green Pond Atlantic salmon (GP AS, $N = 1$), Otsego Lake Atlantic salmon (OL AS, $N = 18$), Cayuga Lake Atlantic salmon (CL AS, $N = 6$), Cayuga Lake brown trout (CL BT, $N = 17$), Seneca Lake brown trout (SL BT, $N = 2$), Seneca Lake lake trout (SL LT, $N = 4$), and Cayuga Lake lake trout (CL LT, $N = 1$). Significance of multiple comparisons are depicted as: 1 = different from SL RT, 2 = different from LCH AS, 3 = different from LC AS, and 4 = different from CL BT ($P \leq 0.05$). The inset depicts the pooled species or stock data as described in the text. Categories from left to right include: SL RT ($N = 22$), SYN[−] Atlantic salmon ($N = 17$), SYN[+] Atlantic salmon ($N = 22$), Finger Lakes (FL) brown trout ($N = 5$), and FL lake trout ($N = 5$). Significance of multiple comparisons depicted in the inset are as follows: a = different from SL RT, b = different from SYN[−] AS, and c = different from SYN[+] AS ($P \leq 0.05$, * $P \leq 0.01$).

previous documentation of Cayuga syndrome in sac fry offspring from female SL salmon, blood levels of thiamine in the immature male SL Atlantic salmon were not different from those of the SYN[−] LC control stocks. Further multiple comparisons revealed that syndrome-afflicted CL, OL, and GP stocks generally had the lowest levels of circulating thiamine, usually significantly lower than those measured in the LCH Atlantic salmon, LC Atlantic salmon, and SL rainbow trout (Figures 1 and 2). Thiamine levels in the blood of brown trout and lake trout from CL and SL were also significantly lower than those from LC (SYN[−]) salmon and SL rain-

brown trout stocks (Figures 1 and 2). Thiamine concentrations in the blood of lake trout from CL and SL were not different from those in SYN[+] Atlantic salmon stocks.

Thiamine Comparisons between Species and Stocks: Pooled Data

If Bonferroni multiple comparisons did not reveal significant differences between stocks of the same species, data were pooled by species and ANOVAs were recomputed, thereby increasing the power of the test. Thus, whole blood data were pooled for each trout and char species, and their thiamine

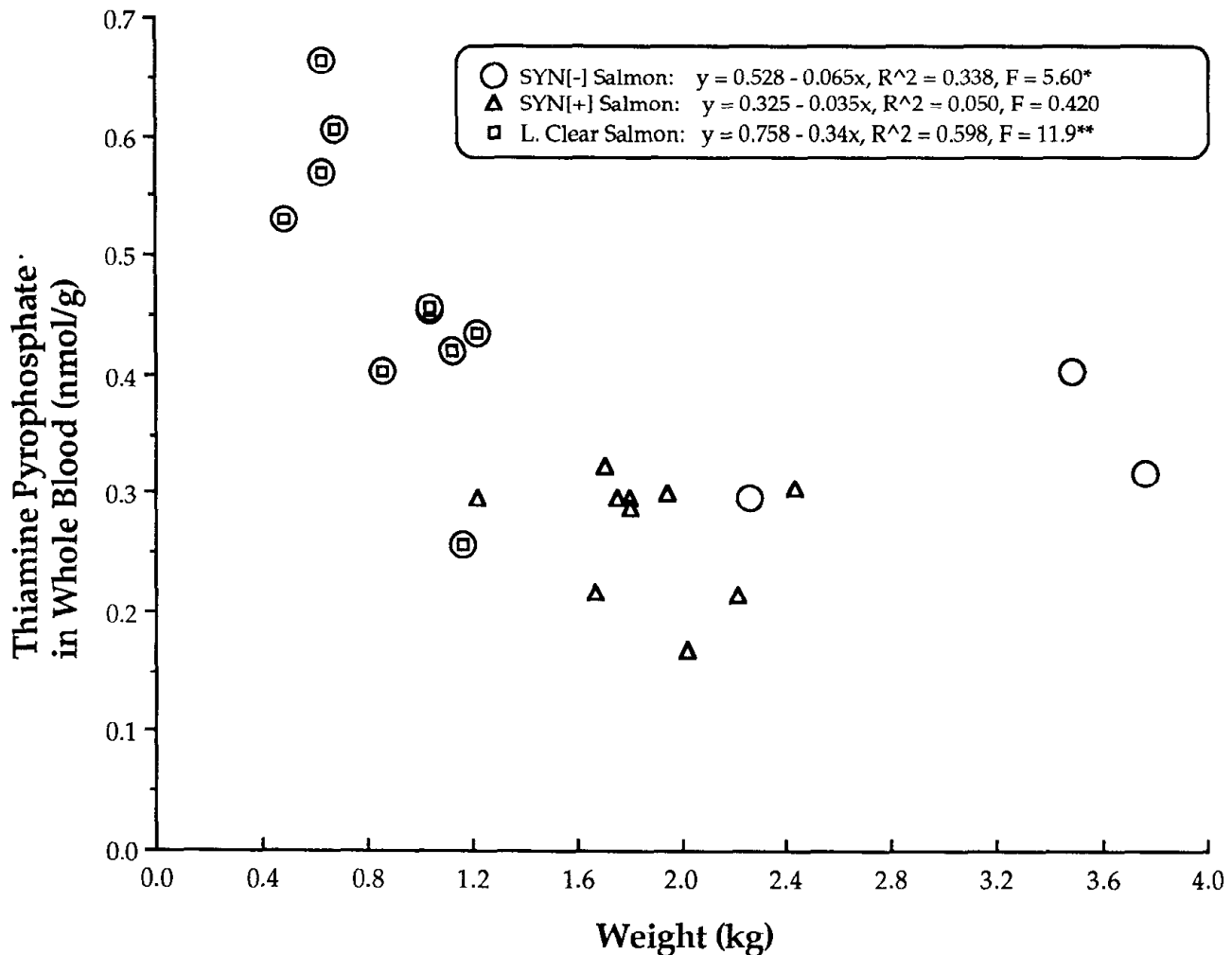


FIGURE 3.—Scatterplots and regressions of whole blood thiamine pyrophosphate versus weight in mature female Atlantic salmon. Little Clear Pond reference control salmon and syndrome-negative Otsego Lake salmon = SYN[-]. Cayuga Lake and syndrome-positive Otsego Lake salmon ($*P \leq 0.05$, $**P \leq 0.01$) = SYN[+].

levels were compared with those found in landlocked salmon that produced either SYN[+] or SYN[-] offspring (Figures 1 and 2, insets).

The ANOVA of the pooled stock data revealed significant differences ($P \leq 0.0001$) between stocks for mean levels of each thiamine moiety ($F_{\log \text{TMP}} = 19.387$, $df = 4, 80$; $F_{\log \text{TPP}} = 24.177$; $F_{\log \text{free thiamine}} = 13.751$; $F_{\log \text{total thiamine}} = 29.068$). The SYN[-] Atlantic salmon had significantly more TPP and total thiamine in their blood than the SYN[+] Atlantic salmon and the FL brown trout and lake trout. The SYN[-] Atlantic salmon also had more TMP than the SYN[+] Atlantic salmon and more free thiamine than all of the other stocks (Figures 1 and 2, insets). Thiamine concentrations in SL rainbow trout exceeded those found in SYN[-] Atlantic salmon for all moieties except free thiamine.

Multiple comparisons of thiamine levels were also performed to isolate differences in thiamine levels between SYN[+] salmon and FL trout and char. Seneca Lake rainbow trout had between twofold and threefold more TMP, TPP, and total thiamine than SYN[+] Atlantic salmon, FL brown trout, and FL lake trout (Figures 1 and 2, insets). Similarly, FL brown trout blood had significantly higher concentrations of TMP, TPP, and total thiamine than the blood from Atlantic salmon with SYN[+] offspring. In contrast, thiamine levels in the blood of FL lake trout and SYN[+] Atlantic salmon did not differ.

Blood and Egg Thiamine versus Maternal Size

Maternal length and weight of the landlocked salmon were inversely related to the blood and egg concentrations of each thiamine moiety. Because TPP was the most concentrated moiety in blood and free thia-

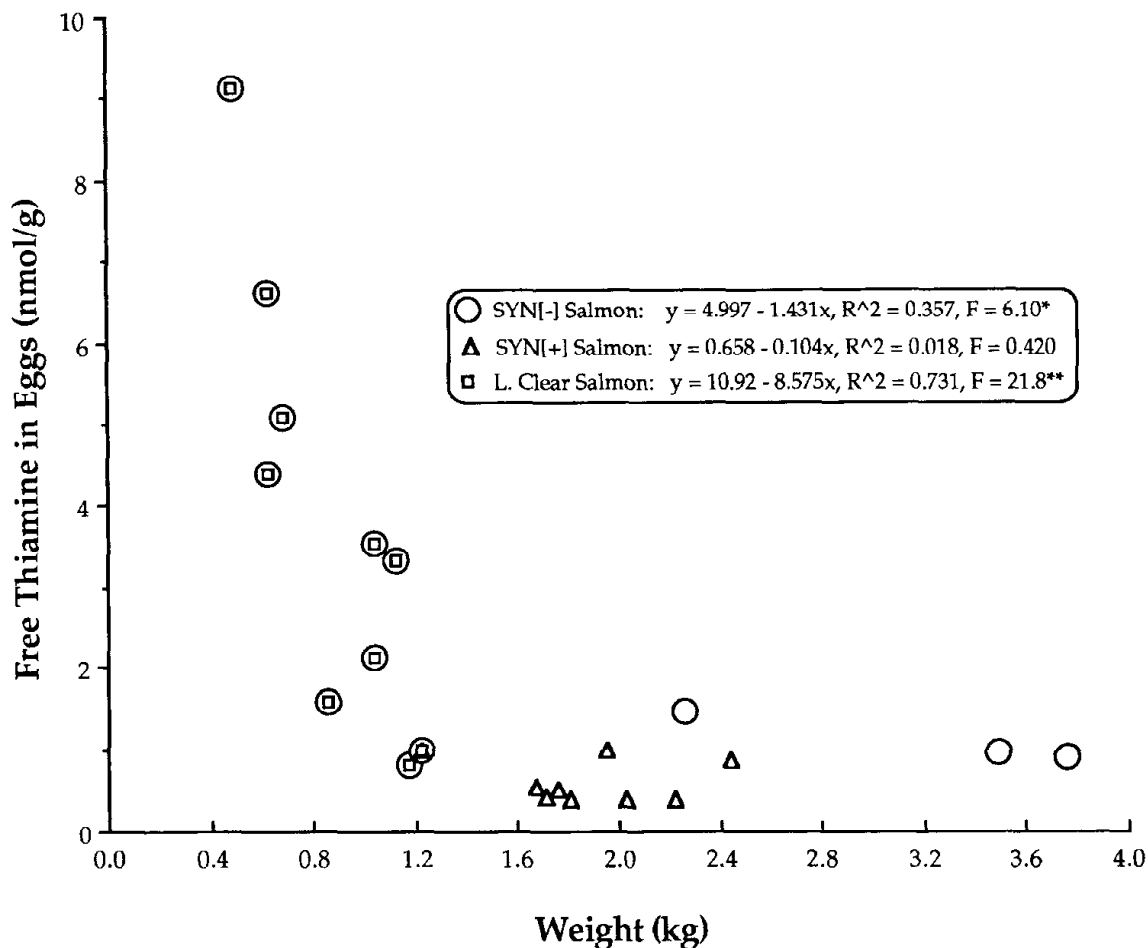


FIGURE 4.—Scatterplots and regressions of egg free thiamine versus weight in mature female Atlantic salmon. Little Clear Pond reference control salmon and syndrome-negative Otsego Lake salmon = SYN[−]. Cayuga Lake and syndrome-positive Otsego Lake salmon (* $P \leq 0.05$, ** $P \leq 0.01$) = SYN[+].

mine was the most concentrated moiety in eggs, only these regression analyses are presented. Similarly, weight was a slightly better predictor of thiamine status than length; thus, only weight data are shown. The graphic relationships identified were somewhat curvilinear, roughly corresponding to the natural groupings of SYN[−] LC salmon (i.e., with or without the three SYN[−] OL salmon) and SYN[+] salmon. For the blood, regressions of TPP versus length and weight were highly significant for salmon with SYN[−] sac fry but insignificant for salmon with SYN[+] sac fry (Figure 3). For the eggs, regressions of free thiamine versus length and weight were highly significant for salmon with SYN[−] sac fry but again insignificant for salmon with SYN[+] sac fry (Figure 4).

Discussion

The current results reveal that whole blood measurements are sufficiently robust to detect substantial interspecies and intraspecies differences in

thiamine status of salmonids that feed to varying degrees on forage species that express thiaminase. Hematocrit and washed packed cell volume were not essential to assess the relationship between blood and egg thiamine profiles in this field-based study. Finger Lakes rainbow trout and brown trout and mature Atlantic salmon that produced SYN[−] sac fry had thiamine levels significantly higher than Atlantic salmon that produced SYN[+] sac fry. Thiamine levels in the blood of SL rainbow trout exceeded those in all other stocks. It was not possible in the current study to monitor the viability of the SL rainbow trout eggs and sac fry. However, total thiamine concentrations in the blood of SL rainbow trout were more than twofold higher than the maternal blood threshold in Atlantic salmon (i.e., 0.44 nmol/g) that prevented syndrome-related mortality in their sac fry progeny (J. P. Fisher, unpublished observations). Given the high levels of thiamine in the SL rainbow trout, the previous demonstration of high viability

in CL rainbow trout (Skea et al. 1985), and the great physical and chemical similarities between SL and CL (Oglesby 1978), it is unlikely that SL rainbow trout suffer mortality akin to the Cayuga or swim-up syndromes.

The current study revealed that TPP was the principal form of thiamine in the blood of all salmonid species investigated, which is in agreement with similar findings in mammals (Combs 1992). Indeed, the relative proportion of the thiamine moieties differed little between the SYN[−] Atlantic salmon, trout, and char species. Given the roles of TPP in bioenergetics (e.g., TPP serves as a critical cofactor in the metabolism of pyruvate and α -ketoglutaric acid), it is understandable that this thiamine moiety predominated in the blood. In SYN[+] salmon, TPP levels were depressed out of proportion with regard to the other moieties. These results indicate that TPP represents the most sensitive indicator of thiamine status in blood and suggest that the quantification of other thiamine moieties may not be necessary for the general screening of thiamine status in the field.

In the current study, evidence that alewife consumption is the cause of depressed thiamine levels was consistent with that presented previously (Fisher et al. 1995a). Salmon from two systems with alewife and marginal (OL) or nonexistent (GP) smelt populations had significantly reduced thiamine levels in their blood. In contrast, the ability of smelt to depress thiamine levels was also suggested by (1) the insignificant difference in thiamine levels in the blood of syndrome-afflicted landlocked salmon (presumed alewife consumption before spawning) and FL lake trout (presumed smelt and alewife consumption), and (2) the significant inverse relationship between growth and thiamine levels in the blood of reference control LC salmon, which presumably increase their consumption of smelt (there is no alewife in LC) as their size increases.

The high levels of thiamine in the blood of SL rainbow trout may reflect the consumption of zooplankton and benthic invertebrates (Jude et al. 1987; N. D. McBride and K. Sanford, NYSDEC, unpublished data, 1996). Such forage species have not been demonstrated to express high thiaminase activity. In turn, predation on thermally partitioned prey reflects the wide temperature tolerance of this species (Haynes et al. 1986). On the basis of Halver's (1989) dietary recommendations for salmonid thiamine requirements (i.e., 33–55 nmol/g), Fitzsimons et al. (1998, this volume) found that Great Lakes invertebrate resources such as *Mysis relicta* and *Diporeia*

species have enough thiamine to prevent deficiencies. It remains to be seen whether these invertebrates, which are also significant forage species for SL rainbow trout, also express thiaminase. Given the high levels of thiamine in the SL rainbow trout and the observations that blood thiamine is rapidly reduced in salmonids fed alewife (Ji et al. 1998, this volume) or the thiamine antagonist amprolium (Fynn-Aikins et al., in press), it is unlikely that the invertebrate prey of salmonids in the FL pose any risk to thiamine nutrition.

The current data showing that brown trout have higher levels of circulating thiamine than Atlantic salmon are also consistent with the hypothesis that brown trout may be eating less thiaminase-rich alewife because of their preference for colder waters within the thermocline (and for the more diversified diet found therein; Jude et al. 1987; Haynes 1995). These data support previous observations of high viability of this species' sac fry in CL and SL (Fisher 1995; Fisher et al. 1995a). Thiamine levels in brown trout from the pooled FL stocks (i.e., FL brown trout in insets to Figures 1 and 2) are, nonetheless, near the survival threshold for maternal blood thiamine in landlocked salmon. Furthermore, despite their elevated levels relative to those of the SYN[+] salmon, they are significantly depressed relative to levels in the SL rainbow trout and the SYN[−] stocks of Atlantic salmon. These data strongly suggest that the FL brown trout are consuming enough thiaminase (probably from a variety of both alewife and smelt) to depress their thiamine levels, but not to a critical level for this species. It is also possible that the brown trout are less sensitive to the effects of thiaminase than the Atlantic salmon.

One of the most surprising findings in the present study was the very low level of thiamine detected in the blood of male SL and CL lake trout. The SL lake trout stock has been captured and cultured routinely for years without documentation of syndrome-related mortality, and the viability of the CL stock was confirmed during earlier studies of the Cayuga syndrome (Fisher 1995). However, in this volume Fitzsimons et al. (1998) report levels of thiamine in SL lake trout eggs of approximately 1.5 nmol/g, less than half the proposed threshold for survival of lake trout swim-up fry from Lake Ontario. Apparently, about 5% swim-up mortality was also seen in the fry from these eggs (K. Osika, NYSDEC, Bath Hatchery, unpublished data). The low levels of thiamine in the blood of male lake trout described here are consistent with the depressed levels seen in the eggs from the subsequent 1995 year-class examined by Fitzsimons et al. (1998).

Several scenarios may explain the low levels of thiamine in SL lake trout blood relative to those found in lower Great Lakes stocks (Fisher et al. 1996b; Fitzsimons et al. 1998). First, the lake trout and/or alewife may be less thermally restricted in SL than they are in Lakes Michigan (Wells 1968; Brandt et al. 1980) and Ontario (Haynes 1995). Thus, they may be eating more alewife than was suggested previously (Fisher et al. 1996b). Second, the lake trout may increase their consumption of smelt in the summer, as shown in Lakes Michigan (Jude et al. 1987) and Ontario (Elrod 1983), but the activity of the smelt thiaminase may be comparable with that of alewife (Ji and Adelman 1998) rather than lower, as demonstrated previously (Gnaedinger 1964). Third, thiamine levels recorded in male lake trout may be erroneously low as a result of their starved, postspawned condition. Finally, factors in the diets of salmonids from the lower Great Lakes, such as contaminants that induce lipid peroxidation (Palace et al. 1998, this volume), may accelerate thiamine metabolism (Lychko et al. 1987) and possibly lead to a higher requirement for the vitamin (Fisher et al. 1995b).

Given the results of numerous studies indicating that smelt are displaced to deeper, colder waters when they are in competition with alewife (Wells 1968; Brandt et al. 1980; Hartman 1988), it seems unlikely that the lake trout in Seneca and Cayuga lakes are consuming only alewife during the critical summer and fall months of oogenesis. In the FL (Youngs and Oglesby 1972), OL (McBride and Sanford, unpublished data), and GP (R. Preall, NYSDEC, region 5, personal communication), salmonid species appear to be vertically stratified only during the summer and fall, when the thermocline is established and stable. Hence, it is conceivable that during periods of thermal stratification, gut content analyses would reveal an increased proportion of smelt and deepwater coregonids in lake trout, a "mixed bag" of smelt, alewife, and midwater invertebrates (e.g., *Mysis* species) in brown trout, and a nearly exclusive diet of alewife in the epilimnetic Atlantic salmon. With this schema, one would also expect little to no interspecies differences in forage items from gut contents of salmonids taken in the winter and spring.

As a preliminary test of this hypothesis, we acquired gut contents from lake trout ($N = 11$), brown trout ($N = 4$), landlocked Atlantic salmon ($N = 3$), and rainbow trout ($N = 2$) from the spring fishing derby on CL mentioned above. Both smelt and ale-

wife were found in the stomachs of each species except rainbow trout (the stomachs of which were empty or the contents unidentifiable), and the smelt was always more digested than the alewife. These early results lend support for the lack of thermal partitioning during the spring. Similar results were obtained by NYSDEC personnel during an April 1990 investigation: both smelt and alewife were found in the stomachs of lake trout, rainbow trout, brown trout, and landlocked salmon. In contrast, only alewife was identified in the stomachs of nine adult (i.e., piscivorous) lake trout (≥ 500 mm) collected in August 1990 (T. Chiotti, NYSDEC, unpublished data), a clear departure from what might be expected from thermal habitat partitioning. More gut content samples are needed from each season to determine whether thermal habitat partitioning can account for the apparent species sensitivity for thiamine deficiency among salmonids in the FL.

Although thiaminase activity has been demonstrated in rainbow smelt (Deutsch and Ott 1942; Deutsch and Hasler 1943; Niellands 1947; Gnaedinger 1964; Ji and Adelman 1998), the ability of smelt diets to produce thiamine deficiency in salmonids under controlled conditions has been equivocal (Wolf 1942; Coble 1965; Ji et al. 1998). The stability of the enzyme in smelt and its ability (or inability) to induce thiamine deficiency in lake trout and other salmonids may be affected, in part, by its digestibility relative to that of alewife and other thiaminase-containing forage species (e.g., buckeye shiner *Notropis atherinoides*; Wolf 1942). Thus, thiaminase activity measured in smelt may be similar to that of alewife, as found by Ji and Adelman (1998), but its functionality *in vivo* may not account for the thiamine deficiency in lake trout. It could be reasoned that a deficiency contributed by smelt is more the result of the lower levels of thiamine in its tissues (Fitzsimons et al. 1998) than of the thiaminase present in its gut.

Perhaps the data that most support a diet-thiaminase connection to reproductive failure in salmonids (be it caused by alewife, smelt, or a combination thereof) comes, surprisingly enough, from the present study, in which blood and egg thiamine levels in the SYN[-] LC Atlantic salmon were regressed against maternal growth indices (Figures 3 and 4). The LC stock lacks an alewife forage, does not show evidence of reproductive impairment (Fisher et al. 1995a, 1995b), and has significantly more blood and egg thiamine than the syndrome-afflicted FL, OL, and GP stocks (Fisher et al. 1996a, 1996b). Yet,

larger LC salmon had substantially less thiamine than smaller LC salmon (Figures 3 and 4). Levels of total thiamine in the sac fry of the LC stock averaged 3.06 nmol/g in the 1994 year-class (Fisher et al. 1996a) and 1.86 nmol/g in the 1993 year-class (Fisher et al. 1996b), both below the threshold of 3.3 nmol/g proposed for lake trout survival (Fitzsimons and Brown 1998). Although smelt are considered the primary forage species of LC Atlantic salmon, populations of golden shiner *Notemigonus crysoleucas*, pumpkinseed sunfish *Lepomis gibbosus*, white sucker *Catostomus commersoni*, and brook trout *Salvelinus fontinalis* are also present (R. Foster, NYSDEC, Adirondack Hatchery, personal communication). Data depicted in Figures 3 and 4 suggest that LC salmon shift their diet as they grow from prey items with low or non-existent thiaminase to the thiaminase-active (and larger) rainbow smelt. This switch to piscivory thus gradually reduces thiamine levels over time; however, reproduction is not impaired in the LC salmon because either they have not targeted smelt for a long enough period to reduce their thiamine intake to a critical level or the smelt diet is not capable of reducing thiamine levels to a great enough degree to cause reproductive impairment, for whatever reason (e.g., digestibility, thiaminase activity, etc.). Similarly, regressions of blood thiamine versus weight or length of SYN[+] salmon were not significant because maternal blood levels were already below the critical threshold necessary for thiamine deposition to occur in the eggs.

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