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Thiamine and Early Mortality Syndrome in Lake Trout[1]

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Abstract.—Reproductive success and vitamin B_1 (thiamine pyrophosphate, thiamine monophosphate, and free thiamine) concentrations were assessed in feral female lake trout *Salvelinus namaycush* from Lake Ontario and Lake Manitou. We monitored fertilization success, survival to hatch, incidence of blue-sac disease, other anomalies, and lake trout early mortality syndrome (EMS). Fertilization and hatching success were high, whereas mortality from blue-sac disease and other anomalies was low in egg batches from both lakes. There was no mortality from EMS in families from Lake Manitou. However, EMS occurred after hatching in the offspring of 48% of the females collected from Lake Ontario. We measured thiamine in liver, red blood cells, eggs, and developing embryos. Relative to fish collected in reference lakes, females in Lake Ontario had depressed hepatic, red blood cell, and egg thiamine concentrations. Although more extensive investigation of thiamine balance is required, it may be possible to use red blood cell thiamine pyrophosphate as a predictive index for EMS susceptibility in offspring. Total thiamine concentrations in developing embryos declined by 50% between fertilization and swim-up. Free thiamine reserves declined most rapidly, whereas levels of thiamine pyrophosphate increased between the eyed embryo and hatch stages. A high proportion (67%) of lake trout families in which the initial egg free thiamine reserves or embryonic concentrations of thiamine pyrophosphate levels were <0.8 nmol/g exhibited EMS. Below this threshold (0.8 nmol/g), the occurrence of EMS was variable (0–100%) and only weakly related to free thiamine concentrations ($r^2 = 0.32$, $P = 0.014$). This observation implies the possibility of additional interactions with other factors.

The presence of maternally transmitted noninfectious ailments that cause yolk sac embryo and swim-up stage mortality has at least partly compromised the sustainability of naturally reproducing populations of salmonids in the lower Great Lakes (Fitzsimons et al. 1995; Marcquenski 1996), the New York Finger Lakes (Fisher et al. 1995), and the Baltic Sea (Johansson et al. 1995). The ailments have been referred to as early mortality syndrome (EMS) in the salmonids from the lower Great Lakes (Marcquenski 1996), Cayuga syndrome in Atlantic salmon *Salmo salar* from the Finger Lakes region (Fisher et al. 1995), and M74 in Baltic Sea salmon (Johansson et al. 1995). One common characteristic of these embryonic mortality syndromes is that treatment of eggs or offspring with prophylactic doses of thiamine alleviates the clinical symptoms (Bylund and Lerche 1995; Fitzsimons 1995). Moreover, analysis of total thiamine levels suggests that low concentrations in eggs are also associated with the ailments (Amcoff et al.1996; Fisher et al.1996).

To enhance understanding of thiamine-related ailments in salmonids, it is essential to determine the cause of the low levels of thiamine and how they relate to developmental processes. Adequate levels of thiamine (particularly the phosphorylated forms) are essential for normal carbohydrate metabolism and neurological function. The diphosphate ester (thiamine pyrophosphate) acts as a cofactor for key enzymatic steps in carbohydrate metabolism (e.g.,

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decarboxylation of α-ketoacids and transketolase reactions), and the phosphorylated forms also play a critical role in nerve function (Combs 1992). Although studies by Fisher et al. (1996) and Amcoff et al. (1996) associate low thiamine concentrations with the observed embryonic mortality in EMS, Cayuga syndrome, and M74, they do not provide sufficient data to assess the roles of the different thiamine forms or to evaluate dose response relationships.

To obtain more information about the role of thiamine in embryonic development, we collected feral female lake trout *Salvelinus namaycush* tissues and gametes from a stock with a history of EMS (Lake Ontario) and an EMS-free stock (Lake Manitou). After fertilization, reproductive competence was assessed by monitoring developmental defects and survival of embryos until swim-up. We analyzed different thiamine forms (thiamine monophosphate, thiamine pyrophosphate, and free thiamine) using high-performance liquid chromatography (HPLC) technology (Brown et al. 1998, this volume). Study objectives were to characterize (1) the prevalence and extent of EMS in lake trout egg batches from Lake Ontario and Lake Manitou; (2) the relationship between thiamine content in females, eggs, and developing embryos; and (3) the association between thiamine content and EMS.

Materials and Methods

Fish and Gamete Collection

Lake trout were captured in Lake Ontario (N = 30) near Port Weller and in Lake Manitou (N = 6) on Manitoulin Island in Lake Huron by small-mesh trap nets set over spawning beds between 20 October and 14 November 1994. Fish were anesthetized in 3-aminobenzoic acid ethyl ester (1:5,000, volume per volume), rinsed in freshwater, and dried. Eggs from ripe fish were expressed into a dry measuring flask and egg volume was recorded. Only females whose eggs showed no evidence of overripening were used. An egg sample (10 g) from each female was placed in a Whirl-Pac® bag and immediately frozen between slabs of dry ice and stored at less than −90°C until analyzed. The remaining eggs were transferred to dry glass jars and placed on ice for transport to the Bayfield Institute laboratory. Semen (1–2 mL), collected from male fish by inserting a glass pipette into the urogenital opening, was placed into dry glass jars and held on ice during transport to the laboratory. After egg collection, blood was removed from the caudal vessels using heparinized 10-mL syringes with 18-gauge needles. Plasma and blood cells were separated by centrifugation and plasma was immediately frozen on dry ice in polyethylene vials. To remove plasma from the red blood cells, they were washed twice by resuspension in isotonic sodium chloride (approximately 300 mosmol/L) and recentrifugation. Fish were killed by a blow to the head and liver tissue was quickly dissected, weighed, and packaged in Whirl-Pac bags. Tissues were immediately frozen between slabs of dry ice and stored at less than −90°C until analyzed. The ages of fish from Lake Ontario were determined using implanted coded wire tags that indicated the date of stocking.

Egg Fertilization and Incubation

Three replicate batches of eggs (20 mL, ≈200 eggs) from each female were fertilized with composite milt (100 μL per 20 mL of eggs) of males (N = 6) from the same lake. Fertilized eggs were water-hardened and reared at 7°C in an incubation facility as described previously (Fitzsimons et al. 1995). A photoperiod of 12 h of light and 12 h of dark was maintained throughout development. Fertilized eggs were examined for developmental anomalies or mortality, and dead eggs or embryos were removed daily. The success of fertilization, hatching deformities, blue-sac disease (Wolf 1957), lake trout EMS, and overall survival were monitored in the offspring of each female. Samples of developing embryos (5–10 individuals) for thiamine analysis were taken at six times after fertilization and placed in sterile vials, quick frozen on dry ice, and stored at less than −90°C until analyzed. To standardize sampling among egg batches collected and fertilized on different dates, sampling occurred at the same cumulative temperature units (CTU; calculated as the incubation temperature in degrees Celsius times the number of days after fertilization) for each egg batch: fertilization (0 CTU), eyed embryo (247 CTU), prehatch embryo (524 CTU), posthatch embryo (686 CTU), pre-swim-up embryo (836 CTU), and post-swim-up embryo (964 CTU).

Thiamine Analysis

Thiamine pyrophosphate, thiamine monophosphate, and thiamine were extracted and quantified by reversed phase HPLC in tissues, eggs, and embryos from each female as described by Brown et al. (1998). Mean assay sensitivity for thiamine and its phosphates was 0.012 pmol. Average recoveries of low and high doses of thiamine compounds added

to tissue samples ranged from 91.4 to 104.5%. Average coefficients of variation for between assay reproducibility ranged from 4.8 to 12.8%.

Statistics

To compare variables based on the presence of EMS in offspring from Lake Ontario females, the measurements were grouped. The first group (LON) represented families ($N = 14$) displaying a low occurrence of EMS (range, 0–4%). The second group (LON-EMS) represented families ($N = 13$) displaying a higher occurrence of EMS (range, 30–97%). Egg batches from three females (two from the LON group and one from the LON-EMS group) exhibited poor fertilization success (<25%). These families had too few survivors to provide accurate estimates for subsequent measurements and were eliminated from the analysis. The Systat statistical package (Wilkinson et al. 1992) was used to analyze the data. Bartlett's test was applied to test for homogeneity of variance and, where necessary, data were log-transformed. Significance was determined by one-way analysis of variance (ANOVA) on means of replicate measures of variables from each female. Because offspring from the same family were sampled at successive times during development, repeated-measures ANOVA (Wilkinson et al. 1992) was used to determine the significance of measured variables in developing offspring. Comparisons of egg diameter were made using analysis of covariance with total body weight adjusted for expressed egg volume (total weight minus expressed egg weight) as the covariate. Comparisons of body weight were made using analysis of covariance with age as the covariate. Pairwise comparisons were conducted by applying the least-significant-difference test to the least-squares means produced by the ANOVAs. Linear relationships between variables were examined by Pearson's product-moment correlation. For all tests, a P-value < 0.05 was considered significant. For clarity of presentation, arithmetic means with standard errors have been used in the tables and figures. A Fulton-type condition factor (Bagenal and Tesch 1978) was calculated as:

$$CF = \frac{100 \times (\text{total fish weight} - \text{expressed egg weight})}{\text{length}^3},$$

where CF = condition factor.

TABLE 1.—Number of fish, size (weight and length), condition, age, and egg size in female lake trout from Lake Manitou (LM), Lake Ontario with a low (<10%) offspring incidence of early mortality syndrome (LON), and Lake Ontario with high (>10%) offspring incidence of EMS (LON-EMS). Values represent mean (SE). Significant differences are indicated ($P < 0.05$) by different letters after the mean. Under Lake Manitou, NA = not analyzed.

Variable	LM	LON	LON-EMS
N	6	14	13
Weight (kg)	2.58 (0.14)	3.63 (0.31)	3.13 (0.21)
Length (cm)	64.0 (0.4)	69.2 (1.6)	67.1 (1.5)
Condition factor	0.99 (0.14)	1.06 (0.15)	1.02 (0.12)
Age (years)	NA	9.9 z (0.8)	7.6 y (0.5)
Egg diameter (mm)	5.53 z (0.05)	5.38 y (0.06)	5.23 y (0.08)

Results and Discussion

Fish Characteristics

Fish from Lake Ontario spanned a range of sizes (1.75–5.55 kg) that did not differ between groups when tested with age as a covariate (Table 1). The fish from Lake Manitou fell within the size range of the Lake Ontario fish, but their sizes could not be compared directly with those of the Lake Ontario groups because aging structures were unavailable. Fish condition was similar among all groups. In Lake Ontario fish, females that produced offspring exhibiting high occurrence of EMS were approximately 2 years younger than those that produced offspring exhibiting low occurrence of EMS. The fish from Lake Manitou produced larger eggs than those from Lake Ontario (Table 1). Egg diameter was related to fish size ($r^2 = 0.251$, $P = 0.013$) in fish from Lake Ontario. However, similar to the observations of Fitzsimons et al. (1995), egg size was unrelated to the presence of EMS in the offspring.

Fertilization and Reproductive Success

Fertilization and survival to hatch did not differ between egg batches, whereas mortality from blue-sac disease and other anomalies was low in egg batches from both lakes (Table 2). There was no mortality attributable to EMS in offspring of Lake Manitou females. Posthatch mortality (>10%) attributable to EMS occurred between 800 and 1,000 CTU

TABLE 2.—Percentage of fertilization, hatching success, blue-sac disease relative to hatch, other anomalies (e.g., bent backs), EMS relative to hatch, and overall survival through 0–1,138 cumulative temperature units in embryos of female lake trout from Lake Manitou (LM), Lake Ontario with a low EMS incidence (LON), and Lake Ontario with a high EMS incidence (LON-EMS). Values represent mean (SE). Significant differences ($P < 0.05$) are indicated by different letters after the mean.

Variable	LM	LON	LON-EMS
Fertilization	86.6 (3.3)	90.2 (2.3)	88.6 (3.2)
Hatching success	73.5 (6.0)	76.2 (6.2)	83.3 (5.3)
Blue-sac disease relative to hatch	4.6 (1.9)	10.9 (4.0)	3.0 (0.8)
Other anomalies	1.4 (0.6)	1.9 (0.9)	0.5 (0.1)
EMS relative to hatch	0	0.9 z (0.8)	71.1 y (6.2)
Overall survival	69.1 z (6.9)	68.6 z (7.9)	20.8 y (5.2)

(Figure 1) and affected offspring from 48% of the females collected from Lake Ontario. The prevalence and clinical signs of EMS (e.g., loss of equilibrium, fish lying on their sides at the bottom of the tank, erratic swimming behavior, hyperexcitability) were similar to those previously reported in lake trout (Fitzsimons et al. 1995). Families suffering significant levels of EMS had low overall survival (Table 2).

Thiamine Levels

In fish from Lake Manitou, thiamine in liver and red blood cells occurred mostly as the metabolically functional enzyme cofactor (thiamine pyrophosphate; Table 3). The hepatic and egg thiamine concentrations of Lake Manitou fish were comparable with those found in lake trout collected at the Experimental Lake Area in northwestern Ontario (Brown et al. 1998), where offspring survival is high (Delorme 1995). Similar concentrations of thiamine pyrophosphate (Table 3) were also observed in juvenile rainbow trout *Oncorhynchus mykiss* fed a commercial diet supplemented with exogenous thiamine (Masumoto et al. 1987). In contrast to the forms found in other tissues, free thiamine was the predominant vitamin form (86%) in eggs of lake trout from Lake Manitou. Significant egg reserves of free

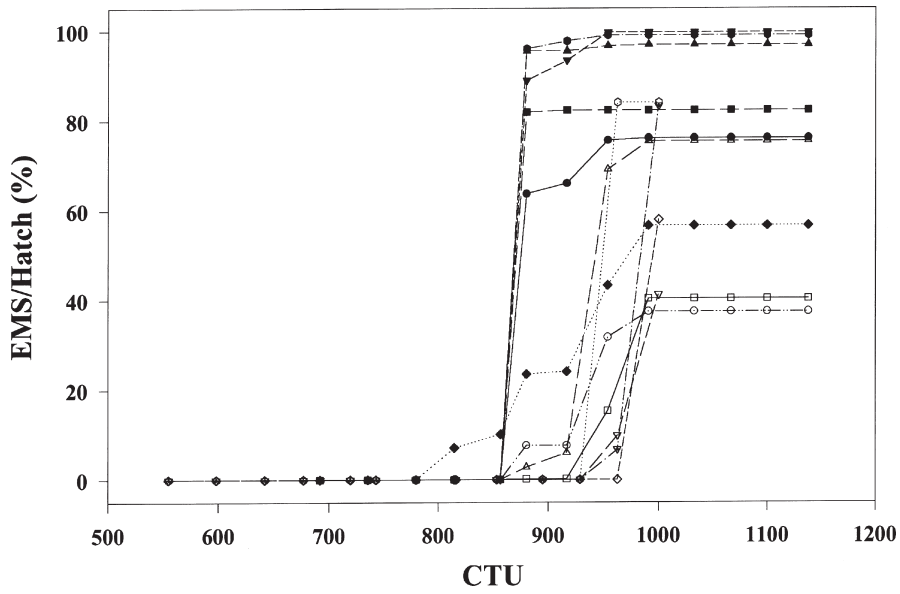

FIGURE 1.—Relationship between mortality as a percentage of the number of progeny hatched from Lake Ontario females ($N = 13$) with high incidence (>10%) of early mortality syndrome (EMS) and cumulative temperature units (CTU) after fertilization. Posthatch mortality attributable to EMS occurred between 800 and 1,000 CTU. Values represent the mean of three replicates of 200 progeny per female.

TABLE 3.—Thiamine pyrophosphate, thiamine monophosphate, and free thiamine levels (nmol/g) in liver and red blood cells of female lake trout from Lake Manitou (LM), Lake Ontario with a low EMS incidence (LON), and Lake Ontario with a high EMS incidence (LON-EMS). Values represent mean (SE) of duplicate measures from 6 (LM), 14 (LON), or 13 (LON-EMS) fish. Significant differences are indicated ($P < 0.05$) by different letters after the mean. For Lake Manitou, NA = not analyzed.

	Thiamine pyrophosphate			Thiamine monophosphate			Free thiamine		
Tissue	LM	LON	LON-EMS	LM	LON	LON-EMS	LM	LON	LON-EMS
Liver	10.19 z	7.00 y	5.98 y	0.34	0.22	0.13	0.31	0.17	0.24
	(0.91)	(0.58)	(0.66)	(0.07)	(0.05)	(0.05)	(0.10)	(0.06)	(0.07)
Red blood cells	NA	0.34 z	0.24 y	NA	0.06	0.07	NA	0.12	0.16
		(0.03)	(0.02)		(0.02)	(0.02)		(0.01)	(0.04)

thiamine are common among lake trout reared on vitamin-fortified hatchery diets or collected from locations where EMS does not occur (Fitzsimons and Brown 1996; Honeyfield et al. 1998, this volume).

Relative to female lake trout collected from Lake Manitou, fish from Lake Ontario had depressed tissue levels of thiamine (Table 3). Hepatic reserves of thiamine pyrophosphate in Lake Ontario females were about 60–70% of those found in fish from Lake Manitou. Red blood cell thiamine pyrophosphate in Lake Ontario fish was 30% that of female fish collected from the Experimental Lake Area (Brown et al. 1998). Moreover, the depressed levels of thiamine found in the lake trout collected from Lake Ontario (Table 3) were comparable with those found in juvenile rainbow trout exhibiting overt signs of thiamine deficiency after consuming a thiamine-deficient diet (Masumoto et al. 1987).

Hepatic concentrations of thiamine pyrophosphate in the Lake Ontario females were unrelated to either the presence of lower egg levels of thiamine or the presence of EMS. However, maternal concentrations of thiamine pyrophosphate in washed red blood cells correlated to the amount of free thiamine ($r^2 = 0.518$, $P < 0.001$) in eggs and to the presence of EMS ($r^2 = 0.252$, $P = 0.009$; Figure 2). When red blood cell thiamine pyrophosphate concentrations were less than 0.33 nmol/g, 63% of females produced offspring exhibiting EMS. Honeyfield et al. (1998) found that different tissue concentrations are not depleted at the same rate when inhibitors of thiamine uptake are fed to fish to produce deficient broodstock and eggs. Although more extensive investigation of thiamine balance is required, it may be possible

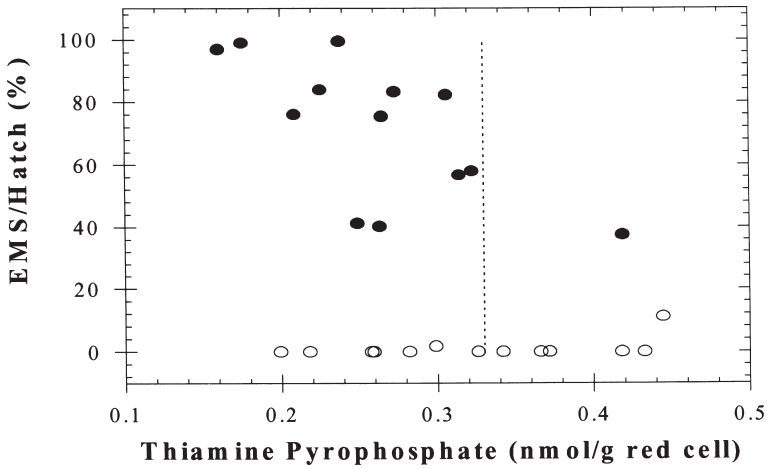

FIGURE 2.—Relationship between thiamine pyrophosphate levels in red blood cells of mothers and incidence of EMS as a percentage of the number of embryos hatched from Lake Ontario females. Symbols indicate values for eggs from Lake Ontario with a low incidence of EMS (○) and with a high incidence of EMS (●). At red blood cell thiamine levels less than 0.33 nmol/g (dotted line), there is an high occurrence of EMS (63%).

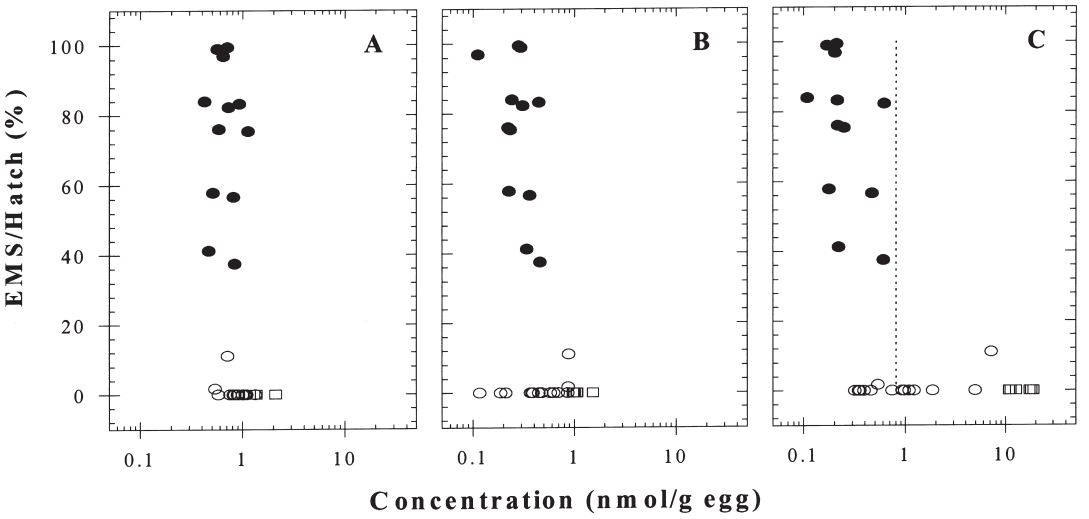

FIGURE 3.—Relationship between incidence of EMS as a percentage of the number of embryos hatched from Lake Ontario females and egg levels of thiamine pyrophosphate (**A**), thiamine monophosphate (**B**), and free thiamine (**C**). Symbols indicate values for eggs from Lake Manitou (□), Lake Ontario with a low incidence of EMS (○), and Lake Ontario with a high incidence of EMS (●). At egg free thiamine levels less than 0.8 nmol/g (dotted line), there is a high occurrence of EMS (67%).

to use red blood cell thiamine pyrophosphate as a predictive index for EMS susceptibility in offspring.

Although all forms of thiamine were lower in eggs of lake trout from Lake Ontario, the levels of free thiamine showed the greatest difference (Table 3). The Lake Ontario fish that produced offspring with a low incidence of EMS had free thiamine levels that were 10% of those of the Lake Manitou fish. Free thiamine levels in eggs produced by females from Lake Ontario whose offspring developed EMS were still lower, 3% of the thiamine levels found in eggs from Lake Manitou females. There was also a shift in thiamine forms: phosphorylated thiamine made up a larger portion of the total thiamine in eggs from Lake Ontario. A high proportion (67%) of lake trout families in which the initial egg free thiamine reserves were <0.8 nmol/g developed EMS (Figure 3A). Below this threshold, the amount of EMS was variable (0–100%) and loosely but significantly related to free thiamine concentrations ($r^2 = 0.32$, $P < 0.05$). Egg concentrations of phosphorylated thiamine forms were unrelated to the amount of EMS (Figure 3, B and C). A similar relationship between egg free thiamine and EMS in developing offspring was observed in coho salmon *Oncorhynchus kisutch* from Lake Michigan (Hornung et al. 1998, this volume). In coho salmon, the threshold egg free thiamine concentration for greater prevalence of EMS was 0.3 nmol/g. Similar to our findings in lake trout, the extent of EMS-related mortality below this threshold concentration in coho salmon eggs was variable and unrelated to further changes in thiamine level (Hornung et al. 1998). The high variability of EMS mortality below a critical threshold level raises the possibility that interactions by factors other than thiamine could also contribute to the development of EMS.

Total thiamine concentrations in developing offspring from all groups declined by 50% between fertilization and swim-up (Table 4). In the only other study of thiamine dynamics in the developing embryo, thiamine concentrations measured by microbial assay in eggs and embryos of hatchery-reared rainbow trout (Sato et al. 1987) were comparable with our findings for total thiamine in the Lake Manitou families. In lake trout, free thiamine reserves declined most extensively in developing embryos (Table 4), whereas levels of thiamine pyrophosphate (active enzyme cofactor) initially declined and then between 247 and 684 CTU (eyed and posthatch embryos; Table 4) increased. It appears that egg free thiamine may serve as a reservoir to supply substrate for production of thiamine pyrophosphate. Thiamine monophosphate levels remained constant during development in the Lake Manitou families (Table 4). In the families from Lake Ontario, thiamine monophosphate declined after hatching but returned to levels found in the freshly fertilized eggs by swim-up.

TABLE 4.—Thiamine pyrophosphate, thiamine monophosphate, and free thiamine levels (nmol/g) in eggs and developing offspring of female lake trout from Lake Manitou (LM), Lake Ontario with a low EMS incidence (LON), and Lake Ontario with a high EMS incidence (LON-EMS) at various stages of development. Values represent mean (SE) of duplicate measures from 6 (LM), 14 (LON), or 13 (LON-EMS) fish. Significant differences between groups are indicated ($P < 0.05$) by different letters after the mean.

	Thiamine pyrophosphate			Thiamine monophosphate			Free thiamine		
Embryonic stage	LM	LON	LON-EMS	LM	LON	LON-EMS	LM	LON	LON-EMS
Fertilized egg	1.29 z	0.90 y	0.70 y	1.10 z	0.51 y	0.30 x	14.85 z	1.53 y	0.39 x
	(0.18)	(0.06)	(0.06)	(0.09)	(0.07)	(0.03)	(1.51)	(0.54)	(0.05)
Eyed embryo	0.80 z	0.27 y	0.24 y	0.98 z	0.39 y	0.41 y	13.73 z	1.31 y	0.39 x
	(0.06)	(0.03)	(0.03)	(0.05)	(0.06)	(0.05)	(0.72)	(0.59)	(0.05)
Prehatch embryo	1.28 z	0.83 y	0.59 y	1.49 z	0.35 y	0.22 x	9.93 z	0.95 y	0.37 x
	(0.12)	(0.07)	(0.03)	(0.01)	(0.04)	(0.02)	(1.06)	(0.25)	(0.05)
Posthatch embryo	5.09 z	1.17 y	0.59 x	1.37 z	0.30 y	0.15 x	5.34 z	0.47 y	0.28 y
	(0.44)	(0.12)	(0.05)	(0.04)	(0.04)	(0.02)	(1.23)	(0.12)	(0.05)
Pre-swim-up embryo	5.63 z	1.05 y	0.56 x	0.73 z	0.25 y	0.02 x	1.40 z	0.17 y	0.07 x
	(0.32)	(0.17)	(0.05)	(0.05)	(0.03)	(0.01)	(0.10)	(0.05)	(0.02)
Post-swim-up embryo	6.63 z	1.10 y	0.47 x	0.91 z	0.29 y	0.08 y	1.30 z	0.12 y	0.05 x
	(0.24)	(0.25)	(0.15)	(0.09)	(0.16)	(0.01)	(0.22)	(0.03)	(0.02)

Posthatch levels of thiamine pyrophosphate less than 0.8 nmol/g corresponded to a high incidence (80%) of EMS in embryos. Further study is required to substantiate the finding that the formation of phosphorylated thiamine in embryos represents a critical step in the development of EMS. However, we note that the elevations in embryonic levels of thiamine pyrophosphate (eyed stage to first feeding) were coincident with the occurrence of rearing losses attributable to early mortality syndrome in the various salmonid species that have been examined (Marcquenski 1996).

Although these results do not explicitly demonstrate cause and effect, it is clear that fish capable of producing eggs and embryos with higher thiamine levels are less likely to exhibit EMS. The high variability in the occurrence of EMS when thiamine concentrations are low implies that other factors may also contribute to the development of EMS. In a companion study (Palace 1996), it was determined that the presence of EMS in offspring of female lake trout from Lake Ontario was unrelated to maternal, egg, or embryo levels of vitamin E, vitamin C, or the carotinoid astaxanthin.

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References

Amcoff, P., L. Norrgren, H. Borjeson, and J. Lindeberg. 1996. Lowered concentrations of thiamine (vitamin B_1) in M74-affected feral Baltic salmon (*Salmo salar*). Pages 38–39 *in* B.-E. Bengtsson, C. Hill, and S. Nellbring, editors. Report from the second workshop on reproduction disturbances in fish. Swedish Environmental Protection Agency Report 4534, Stockholm.

Bagenal, T. B., and F. W. Tesch. 1978. Age and growth. Pages 101–136 *in* T. Bagenal, editor. Methods for assessment of fish production in fresh waters, 3rd edition. Blackwell Scientific Publications, Oxford, UK.

Brown, S. B., D. C. Honeyfield, and L. Vandenbyllaardt. 1998. Thiamine analysis in fish tissues. Pages 73–81 *in* G. McDonald, J. D. Fitzsimons, and D. C. Honeyfield, editors. Early life stage mortality syndrome in fishes of the Great Lakes and Baltic Sea. American Fisheries Society, Symposium 21, Bethesda, Maryland.

Bylund, G., and O. Lerche. 1995. Thiamine therapy of M74 affected fry of Atlantic salmon, *Salmo salar*. Bulletin of the European Association of Fish Pathologists 15(3):93–97.

Combs, G. F. 1992. The vitamins. Academic Press, San Diego, California.

Delorme, P. D. 1995. The effect of toxaphene, chlordane and 2,3,4,7,8-pentachlorodibenzofuran on lake trout and white sucker in an ecosystem experiment and the distribution and effects of 2,3,4,7,8-pentachlorodibenzofuran on white suckers and broodstock rainbow trout in laboratory experiments. Doctoral dissertation. University of Manitoba, Winnipeg.

Fisher, J. P., J. D. Fitzsimons, G. F. Combs, and J. M. Spitzbergan. 1996. Naturally occurring thiamine deficiency causing reproductive failure in Finger Lakes Atlantic salmon and Great Lakes lake trout. Transactions of the American Fisheries Society 125:167–178.

Fisher, J. P., and six coauthors. 1995. Reproductive failure of landlocked Atlantic salmon from New York's Finger Lakes: investigation into etiology and epidemiology of the "Cayuga syndrome." Journal of Aquatic Animal Health 7:81–95.

Fitzsimons, J. D. 1995. The effect of B-vitamins on a swim-up syndrome in Lake Ontario lake trout. Journal of Great Lakes Research 21 (Supplement 1):286–289.

Fitzsimons, J. D., and S. B. Brown. 1996. Effect of diet on thiamine levels in Great Lakes lake trout and relationship with early mortality syndrome. Pages 76–78 *in* B.-E. Bengtsson, C. Hill, and S. Nellbring, editors. Report from the second workshop on reproduction disturbances in fish. Swedish Environmental Protection Agency Report 4534.

Fitzsimons, J. D., S. Huestis, and B. Williston. 1995. Occurrence of a swim-up syndrome in Lake Ontario lake trout in relation to contaminants and cultural practices. Journal of Great Lakes Research 21 (Supplement 1):277–285.

Honeyfield, D. C., K. Fynn-Aikins, J. D. Fitzsimons, and J. A. Mota. 1998. Effect of dietary amprolium on egg and tissue thiamine concentrations in lake trout. Pages 172–177 *in* G. McDonald, J. D. Fitzsimons, and D. C. Honeyfield, editors. Early life stage mortality syndrome in fishes of the Great Lakes and Baltic Sea. American Fisheries Society, Symposium 21, Bethesda, Maryland.

Hornung, M. W., L. Miller, R. E. Peterson, S. Marcquenski, and S. B. Brown. 1998. Efficacy of thiamine, astaxanthin, β-carotene, and thyroxine treatments in reducing early mortality syndrome in Lake Michigan salmonid embryos. Pages 124–134 *in* G. McDonald, J. D. Fitzsimons, and D. C. Honeyfield, editors. Early life stage mortality syndrome in fishes of the Great Lakes and Baltic Sea. American Fisheries Society, Symposium 21, Bethesda, Maryland.

Johansson, N., P. Jonsson, O. Svanberg, A. Sodergren, and J. Thulin. 1995. Reproduction disorders in Baltic fish. Swedish Environmental Protection Agency Report 4347, Stockholm.

Marcquenski, S. V. 1996. Characterization of early mortality syndrome (EMS) in salmonids from the Great Lakes. Pages 73–75 *in* B.-E. Bengtsson, C. Hill, and S. Nellbring, editors. Report from the second workshop on reproduction disturbances in fish. Swedish Environmental Protection Agency Report 4534, Stockholm.

Masumoto, T., R. W. Hardy, and E. Casillas. 1987. Comparison of transketolase activity and thiamine pyrophosphate levels in erythrocytes and liver of rainbow trout (*Salmo gairdneri*) as indicators of thiamine status. Journal of Nutrition 117:1422–1426.

Palace, V. P. 1996. Oxidative stress in lake trout (*Salvelinus namaycush*) exposed to organochlorine contaminants that induce phase I biotransformation enzyme system. Doctoral dissertation. University of Manitoba, Winnipeg.

Sato, M., R. Yoshinaka, R. Kuroshima, H. Morimoto, and S. Ikeda. 1987. Changes in water soluble vitamin contents and transaminase activity of rainbow trout egg during development. Nippon Suisan Gakkaishi 53:795–799.

Wilkinson, L., M. Hill, J. P. Welna, and G. K. Birkenbeuel. 1992. Systat for Windows: statistics version 5. Systat Inc. Evanston, Illinois.

Wolf, K. 1957. Blue-sac disease investigations: microbiology and laboratory induction. Progressive Fish-Culturist 19:14–18.