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Effect of Dietary Amprolium on Egg and Tissue Thiamine Concentrations in Lake Trout

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Abstract.—Dietary amprolium, a thiamine antagonist, was fed to lake trout *Salvelinus namaycush* broodstock from April to October before spawning to determine its effect on egg and tissue concentrations of thiamine, thiamine monophosphate, and thiamine pyrophosphate. The thiamine concentration of eggs from fish fed no amprolium was 61.8 nmol/g, whereas the concentration of thiamine in fish fed 0.05 and 0.10% amprolium was 4.02 and 1.71 nmol/g ($P < 0.01$), respectively. In lake trout fed 0.10% amprolium beginning in August, egg free thiamine concentration was reduced to 11.6 nmol/g. No sign of early mortality syndrome was observed in sac fry from eggs in this study, which suggests that thiamine concentrations in the egg were not low enough to be below a critical threshold or that factors other than thiamine are involved in early mortality syndrome.

The reproductive success of Great Lakes lake trout *Salvelinus namaycush*, coho salmon *Oncorhynchus kisutch*, chinook salmon *O. tshawytscha*, and some strains of steelhead trout *O. mykiss*, as well as Atlantic salmon *Salmo salar* in the New York Finger Lakes (Cayuga Lake) and the Baltic Sea, has markedly declined since 1992 because of an increase in a particular type of larval mortality. This larval mortality, which occurs between hatch and swim-up, has been called swim-up syndrome in Great Lakes lake trout (Fitzsimons et al. 1995), Cayuga syndrome in Finger Lakes Atlantic salmon (Fisher et al. 1995, 1996), and M74 in Baltic Sea Atlantic salmon (Bengtsson et al. 1994; Norrgren 1994; Johansson et al. 1995). The clinical signs of affected species include erratic swimming, hyperexcitability, dark coloration, lethargy, anemia, emaciation, and anorexia before death. Recent data reported for Lake Michigan (Marcquenski and Brown 1997) indicate fry mortality of 70% for coho salmon, 60% for chinook salmon, 35% for steelhead trout, and 80% for lake trout; these rates have increased from less than 30% before 1990 (Simonin et al. 1990). Many questions remain about the etiology of early mortality syndrome (EMS), but low egg thiamine concentrations have been linked with the various mortality syndromes.

Total thiamine concentration in lake trout eggs collected from lakes with early life stage mortality (Fitzsimons and Brown 1995) were 1.28 nmol/g for Charleston Lake, 2.25 nmol/g for Lake Ontario, 3.2 nmol/g for Lake Erie, and 3.4 nmol/g for Lake Michigan. In the same report, egg thiamine concentrations were higher in lakes where EMS was absent: 9.0 nmol/g for Lake Simcoe, 14.7 nmol/g for Lake Superior, 15.1 nmol/g for Lake Manitou, 25.3 nmol/g for Lake 375, and 32.1 nmol/g for Lake 442. Similarly, in Lake Michigan coho salmon, three studies have reported low egg thiamine concentrations in groups exhibiting high incidence of EMS (1.2–1.9 nmol/g, Marcquenski and Brown 1997; less than 0.9 nmol/g, Hornung et al. 1995; and 1.1–1.8 nmol/g, Honeyfield et al. 1998, this volume). Fry loss was absent or minimal in coho eggs from Lake Michigan

with total thiamine concentrations greater than 0.9 nmol/g (Hornung et al. 1995) and 2.1–6.3 nmol/g (Honeyfield et al. 1998) and in coho eggs from Lake Superior with concentrations of 65 nmol/g (Marcquenski and Brown 1997). Finally, studies with Baltic Sea salmon (Amcoff et al. 1995) and Atlantic salmon (Fisher et al. 1996) reported high incidences of fry loss when total egg thiamine was less than 1.0 nmol/g. These data suggest that there is a critical threshold concentration for the onset of EMS.

Fry survival can be improved by water hardening eggs or immersing sac fry in a thiamine solution (Bylund and Lerche 1995; Koski et al. 1995; Fisher et al. 1996). Although these results do not constitute evidence of a cause and effect relationship, they do indicate that thiamine likely plays an important role in the development of EMS.

In related research, other water-soluble vitamins and thyroxine gave variable results, but they were generally less effective than thiamine in the treatment of EMS. Fitzsimons (1995) found that riboflavin, folic acid, nicotinic acid, and pyridoxine had no beneficial effect. In contrast, Hornung et al. (1995) found that treatment of fry with 2 mg/L thyroxine reduced EMS-related mortality from 40 to 17%. J. Hnath (Michigan Department of Natural Resources, personal communication) found a 40% improvement in fry survival of Lake Michigan chinook salmon by treating fry in an immersion bath of vitamin C (100–1,000 mg/L). The metabolic functions of thyroxine and ascorbic acid are different from that of thiamine. Therefore, it remains to be determined if the beneficial effects of thyroxine and ascorbic acid can be substantiated by others and shown to be directly related to EMS. One hypothesis for the cause of low concentrations of thiamine in eggs concerns a thiamine-destroying enzyme, thiaminase, in forage fish. Rainbow smelt *Osmerus mordax* and alewife *Alosa pseudoharengus* are forage fishes of salmonids and are known to contain high levels of thiaminase (Neilands 1947; Gnaedinger and Krzeczkowski 1966). Among the Great Lakes, the occurrence of EMS is high in Lakes Michigan and Ontario, low in Lake Erie (Fitzsimons et al. 1995), and nonexistent in Lakes Superior and Huron (Mac et al. 1985). In the New York Finger Lakes, the syndrome afflicts Atlantic salmon from Cayuga, Keuka, and Seneca lakes, all of which have alewife and smelt populations (Fisher et al. 1996).

TABLE 1.—Composition of experimental diets.

Ingredient	Composition (%)		
	Diet 1	Diet 2	Diet 3
Herring meal (70%)	32.0	32.0	32.0
Corn gluten meal	18.0	18.0	18.0
Blood flour	8.6	8.6	8.6
Herring oil	8.0	8.0	8.0
Dextrin	30.0	29.95	29.9
Choline chloride	0.5	0.5	0.5
Vitamin premix[a]	0.5	0.5	0.5
Mineral premix[b]	0.2	0.2	0.2
Ascorbic acid	0.2	0.2	0.2
Ameribond, pellet binder	2.0	2.0	2.0
Amprolium chloride	0.0	0.05	0.1

[a] Vitamin premix supplied per kilogram of diet: vitamin A, 3,297 IU; vitamin D, 220 IU; vitamin E, 175 IU; vitamin K, 5.5 mg; ascorbic acid, 330 mg; biotin, 180 mg; vitamin B_{12}, 10 mg; folic acid, 4.4 mg; niacin, 110 mg; pantothenate, 50 mg; pyridoxine, 15.5 mg; riboflavin, 30 mg; thiamine, 17.6 mg (diet 1 only).

[b] Mineral premix (Bernhart-Tomarelli, ICN Nutritional Biochemical, Cleveland, Ohio) supplied per kilogram of diet: calcium carbonate, 630 mg; calcium phosphate, 22,050 mg; citric acid, 68.1 mg; cupric citrate-2½ H_2O, 13.8 mg; ferric citrate-5H_2O, 1,674 mg; magnesium oxide, 750 mg; manganese citrate, 250.5 mg; potassium iodide, 0.3 mg; potassium phosphate dibasic, 2,430 mg; potassium sulfate, 2,040 mg; sodium chloride, 918 mg; sodium phosphate, 64.2 mg; zinc citrate-2H_2O, 39.9 mg.

In contrast, fish from Skaneateles Lake, which has neither smelt nor alewife, are not afflicted with EMS. Thus, Fisher et al. (1996) have proposed that EMS is a result of fishes consuming alewives and smelt.

Mac et al. (1985) suggested that EMS in lake trout is caused by contaminants; however, no direct cause and effect connection has been demonstrated (Fitzsimons et al. 1995). Furthermore, the incidence of EMS has increased while levels of organochlorine and heavy metal toxicants that are widely measured in fish flesh have decreased (Leatherland 1993). Lesser known contaminants or their metabolites have not been ruled out; of particular concern are those compounds that act on the endocrine system. Exposure of fishes to endocrine-disrupting compounds has been associated with a decrease in fertility (Moccia et al. 1986; Leatherland 1993, 1994; Hontela et al. 1995). One conclusion that may be drawn is that

TABLE 2.—Mean levels of thiamine pyrophosphate (TPP), thiamine monophosphate (TP), and free thiamine (T) in eggs of lake trout fed three levels of amprolium. Means with the same letters in the same column are not significantly different ($P > 0.01$).

Diet	Mean egg concentration			
	TPP	TP	T	*N*
Diet 1, 0% amprolium	0.29 z	0.35 z	61.78 z	12
Diet 2, 0.05% amprolium	0.25 yz	0.34 z	4.02 y	9
Diet 3, 0.10% amprolium	0.15 y	0.20 z	1.71 y	6
Diet 3, 0.10% amprolium (Aug)	0.13 y	0.27 z	11.59 y	9
Pooled SE	0.02	0.04	4.49	

if contaminants are involved in the etiology of EMS, and given the fact that there is an absence of typical signs and pathology of acute or chronic toxicity, then the role of contaminants is through an unidentified mechanism.

Early mortality syndrome has not previously been experimentally induced in broodstock under laboratory conditions. Experimental injection of healthy lake trout fry with pyrithiamine, a thiamine antagonist, has resulted in fry mortality resembling that of EMS (J. D. Fitzsimons, Bayfield Institute, personal communication). Thiamine deficiency has been reported in carp *Cyprinus carpio* and rainbow trout (nonanadromous *Oncorhynchus mykiss*) fed diets containing amprolium, a thiamine antagonist (Aoe et al. 1969). A means of reproducing EMS in the laboratory would facilitate the search for the cause of EMS. Therefore, the objectives of this study were to evaluate the ability of amprolium to reduce egg thiamine concentrations in lake trout and to determine if EMS symptoms could be produced in fry hatched from eggs of lake trout fed amprolium.

Materials and Methods

Diets were formulated with 29–30% dextrin to be high in carbohydrates (Table 1) to increase the metabolic demand for thiamine. Amprolium was supplemented at three concentrations (0, 0.05, and 0.10%). Dietary ingredients were chosen to produce a low-thiamine feed. First time spawning 4-year-old lake trout were allocated to one of four dietary treatments. Fish on three of the four treatments were fed the basic test diet with 0, 0.05, or 0.10% amprolium added, beginning 1 April 1995 through spawning in October and November 1995. Fish in the fourth treatment group were fed a commercial broodstock feed (Zeigler Brothers, Inc., Gardners, Pennsylvania) from April through July followed by diet 3 containing 0.10% amprolium from 1 August through spawning. The delay in administration of amprolium to the fourth group was to correspond with an expected surge in vitellogenesis. Therefore the fourth treatment was to determine the effect of amprolium on egg thiamine concentrations when amprolium was given only during the latter stages of egg maturation.

Adult lake trout (Seneca strain) were maintained in 2.74-m circular tanks adjacent to large windows inside the fish culture laboratory. All aspects of physical behavior, number of fish spawning, and number of eggs per female in the experimental fish were the same as in fish maintained in outside facilities. Standard hatchery practices for salmonids (Piper et al. 1982) were used for spawning, fertilization, and egg incubation. Eggs were incubated in Heath trays. Well water with a constant temperature of 9°C was used throughout the study.

Tissue, red blood cell, and egg concentrations of free thiamine, thiamine monophosphate, and thiamine pyrophosphate were determined using the high-pressure liquid chromatography method of Brown et al. (1998, this volume). Blood samples were collected on 15 June, 28 July, and at spawning (October–November). Fish were sedated with tricaine methanesulfonate (MS-222) and blood was collected from the tail vein in heparinized syringes. Blood was centrifuged to remove the plasma. Red blood cells were resuspended in normal saline and centrifuged twice. Washed cells were stored frozen (−80°C). At spawning, four fish per treatment group were killed using an overdose of MS-222. Tissue was immediately collected and stored frozen (−80°C) for analysis of thiamine concentrations. Eggs from 6 to 12 fishes were collected from each treatment group and an aliquot of their eggs was incubated. The incidence of EMS in the fry was monitored from hatching through swim-up by visual observation for symptoms. The remaining unfertilized eggs were stored frozen for thiamine analysis. Data were analyzed as a completely randomized design using the General

TABLE 3.—Mean levels of thiamine pyrophosphate (TPP), thiamine monophosphate (TP), and free thiamine (T) in tissues of lake trout fed three levels of amprolium. Means with the same letter in the same column are not significantly different ($P > 0.01$).

	Mean tissue concentration (nmol/g of wet tissue)											
	Liver			Muscle			Kidney			Heart		
Diet	TPP	TP	T	TPP	TP	T	TPP	TP	T	TPP	TP	T
Diet 1[a]	5.32 z	2.59 yz	1.80 z	4.10 z	0.80 z	0.35 yz	2.15	1.51	1.07	41.20 z	11.01 z	4.37 z
Diet 2[b]	3.68 yz	2.88 yz	1.02 y	1.93 y	0.44 y	0.35 yz	2.40	1.69	2.14	15.20 x	3.57 y	0.92 y
Diet 3[c]	3.33 y	1.83 y	1.51 yz	1.01 y	0.23 y	0.31 y	1.57	1.09	0.50	9.45 x	3.14 y	1.59 y
Diet 3[c] (Aug)	4.50 yz	3.59 z	1.70 yz	3.90 z	0.83 z	0.38 z	1.50	1.11	1.04	24.38 y	5.24 y	1.01 y
Pooled SE	0.32	0.24	0.15	0.37	0.07	0.01	0.25	0.16	0.27	3.51	0.96	0.41

[a] Diet 1, 0% amprolium.
[b] Diet 2, 0.05% amprolium.
[c] Diet 3, 0.10% amprolium.

Linear Model of SAS for means and analysis of variance (SAS 1994). Differences among means were separated using Duncan's new multiple-range test (SAS 1994).

Results and Discussion

This study with lake trout and an associated study with Atlantic salmon (Fynn-Aikins et al., in press) constitute steps toward developing a laboratory model for the study of EMS. The data show that dietary amprolium is an effective thiamine antagonist that reduces the deposition of thiamine into the egg.

Free thiamine is the predominant form of thiamine found in lake trout eggs (Table 2). Fish fed no amprolium had higher levels of free thiamine in eggs than fish fed either 0.05 or 0.10% amprolium. Lake trout fed 0.10% amprolium beginning in August (versus April) also had significantly lower thiamine levels than the controls. In August there is a significant increase in estrogen-stimulated vitellogenesis in salmonids (Crim and Idler 1978). In addition, estrogen stimulates the synthesis of a thiamine carrier protein in avian and amphibian species, and it has been postulated that this estrogen-stimulated thiamine carrier protein is specific for the transport of thiamine into the eggs of fish as well (Adiga and Murty 1983). The results from fish fed amprolium starting in August are consistent with the hypothesis that a carrier protein exists. Thiamine values for the group fed amprolium beginning in August were more variable than those observed in the other treatment groups. Free thiamine values ranged from 1.36 to 46.52 nmol/g of egg in the August group, and if the highest two values are removed from the data, the mean changes from 11.59 to 5.56 nmol/g. This latter mean is numerically similar to that found with diet 2, which suggests that feeding lake trout broodstock a thiamine antagonist during the latter phase of egg development will result in lower, but highly variable, egg thiamine levels among individual fishes.

In contrast to the egg data, the predominant form of thiamine found in liver, muscle, kidney, and heart (Table 3) was thiamine pyrophosphate (TPP), which is the active form or the cofactor in thiamine-containing enzymes (Gubler 1991). Heart tissue from fish fed no amprolium contained the highest level of TPP, followed by liver and muscle. There was a decrease in TPP concentration in these three tissues when fish were fed diets containing 0.10% amprolium from April through spawning. Muscle TPP was similar between the groups fed no amprolium and those fed 0.10% amprolium beginning in August. Lake Michigan coho salmon with high, medium, and low incidences of EMS showed no differences in muscle TPP concentrations (Honeyfield et al. 1998). When Atlantic salmon were fed three dietary levels of amprolium, as in the present lake trout study (April through spawning), no differences were observed in muscle TPP (Fynn-Aikins et al., in press). In contrast, lake trout fed a thiamine antagonist beginning in April had lower muscle concentrations of thiamine. It appears that lake trout stores of thiamine are more labile than those of Atlantic salmon and possibly coho salmon muscle.

The effect of amprolium on red blood cell (RBC) concentration of thiamine (Table 4) was measurable at first sampling (15 June). Total thiamine and TPP in RBC were reduced as dietary levels of amprolium increased ($P < 0.05$). Free thiamine and thiamine monophosphate levels in RBC from fish fed 0.10% amprolium were lower than those from fish fed no amprolium. Total thiamine concentration in RBCs decreased from June through spawning with all three

TABLE 4.—Mean concentrations of thiamine pyrophosphate (TPP), thiamine monophosphate (TP), and free thiamine (T) in red blood cells of lake trout fed three levels of amprolium. Means with the same letters in the same column within a sampling period are not significantly different ($P > 0.05$).

Diet	Red blood cell concentration (nmol/g of wet tissue) TPP	TP	T	Total thiamine
		15 June		
Diet 1[a]	1.34 z	0.87 z	0.33 z	2.54 z
Diet 2[b]	0.66 y	0.22 y	0.12 yz	0.99 y
Diet 3[c]	0.39 x	0.16 y	0.03 y	0.58 x
Pooled SE	0.150	0.085	0.130	0.191
		28 July		
Diet 1[a]	0.14 z	0.91 z	0.35 z	1.39 z
Diet 2[b]	0.08 y	0.29 y	0.42 y	0.78 y
Diet 3[c]	0.08 y	0.19 y	0.45 x	0.72 y
Pooled SE	0.020	0.223	0.015	0.225
		Spawning (Oct–Nov)		
Diet 1[a]	0.22 z	0.24 z	0.17 z	0.63 z
Diet 2[b]	0.15 yz	0.11 y	0.15 zy	0.40 y
Diet 3[c]	0.08 y	0.08 y	0.14 y	0.30 y
Pooled SE	0.069	0.046	0.015	0.117

[a] Diet 1, 0% amprolium.
[b] Diet 2, 0.05% amprolium.
[c] Diet 3, 0.10% amprolium.

dietary treatments. We surmise that the lowest levels observed at spawning were related to the fasting state of the fish. Feed was offered daily up to 2 weeks before spawning, although feed intake decreased in early September and all feeding ceased by mid to late October, or approximately 1 month before spawning. From this we concluded that RBC thiamine concentrations are affected by amprolium and are a function of time. However, in nonreproductive fish, RBC TPP concentrations of young rainbow trout did not decrease during a 30-week study (Masumoto et al. 1987). Thus, the use of a single RBC thiamine measurement cannot reliably determine thiamine status in reproductive lake trout.

No EMS deaths were observed in fry of lake trout in the present study. The lack of signs of EMS is in contrast to our Atlantic salmon study, in which EMS was observed (Fynn-Aikins et al., in press). In both studies, egg concentration of thiamine was reduced with amprolium, demonstrating that amprolium is an effective experimental tool for reducing egg thiamine levels. The presence of fry mortality in Atlantic salmon and not in lake trout suggests that species differences exist or that multiple factors interact to cause EMS.

General observations about the adult lake trout fed amprolium are unremarkable. Spawning, health, and physical appearance were similar to those of lake trout not fed experimental diets. The only observation that can be made about fry from fish fed experimental diets with or without amprolium is that the fry tended to be slower to swim-up by 3–5 d compared with fry from fish fed commercial feed. This did not appear to be related to EMS. Otherwise, no signs or symptoms of EMS were observed.

References

Adiga, P. R., and C. V. R. Murty. 1983. Vitamin carrier proteins during embryonic development in birds and mammals. Pages 111–136 *in* R. Porter and J. Whelan, editors. Molecular biology of egg maturation. Pitman Books, London.

Amcoff, P., L. Norrgren, H. Borjeson, and J. Lindeberg. 1995. Lowered concentrations of thiamine (vitamin B_1) in M74-affected feral Baltic salmon (*Salmo salar*). Pages 38–39 *in* Bengtsson et al. (1995).

Aoe, H., and five coauthors. 1969. Water-soluble vitamin requirements of carp - VI. Requirement for thiamine and effects of antihistamines. Bulletin of the Japanese Society of Scientific Fisheries 35:459–465.

Bengtsson, B.-E., C. Hill, and S. Nellbring, editors. 1995. Report from the second workshop on reproduction disturbances in fish. Swedish Environmental Protection Agency Report 4534, Stockholm.

Bengtsson, B. E., and six coauthors. 1994. Reproductive disturbances in Baltic fish. Swedish Environmental Protection Agency Report 4319, Stockholm.

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.

Crim, L. W., and D. R. Idler. 1978. Plasma gonadotropin, estradiol and vitellogenin and gonad phosvitin levels in relation to the seasonal reproductive cycles of female brown trout. Annales de Biologie Animale Biochimie Biophysique 18:1001–1005.

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

Fisher, J. P., and six coauthors. 1995. Reproductive failure in landlocked Atlantic salmon from New York's Finger Lakes: investigations into the etiology and epidemiology of the "Cayuga syndrome." Journal of Aquatic Animal Health 7:81–94.

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., and S. Brown. 1995. Effect of diet on thiamine levels in Great Lakes Lake trout and relationship with early mortality syndrome. Pages 76–78 *in* Bengtsson et al. (1995).

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.

Fynn-Aikins, K., P. R. Bowser, D. C. Honeyfield, J. D. Fitzsimons, and H. G. Ketola. In press. Effect of dietary amprolium on tissue thiamin and Cayuga syndrome in Atlantic salmon. Transactions of the American Fisheries Society.

Gubler, C. J. 1991. Thiamin. Pages 233–280 *in* L. J. Machlin, editor. Handbook of vitamins, second edition. Marcel Dekker, New York.

Gnaedinger, R. H., and R. A. Krzeczkowski. 1966. Heat inactivation of thiaminase in whole fish. Commercial Fisheries Review 28(8):11–14.

Honeyfield, D. C., J. G. Hnath, J. Copeland, K. Dabrowski, and J. H. Blom. 1998. Correlation of nutrients and environmental contaminants in Lake Michigan coho salmon with incidence of early mortality syndrome. Pages 135–145 *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.

Hontela, A., P. Dumont, D. Duclos, and R. Fortin. 1995. Endocrine and metabolic dysfunction in yellow perch, *Perca flavescens*, exposed to organic contaminants and heavy metals in the St. Lawrence River. Environmental Toxicology and Chemistry 14:725–731.

Hornung, M. W., L. Miller, R. E. Peterson, S. V. Marcquenski, and S. Brown. 1995. Evaluation of nutritional and pathogenic factors in early mortality syndrome in Lake Michigan salmonids. Pages 82–83 *in* Bengtsson et al. (1995).

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

Koski, P., M. Pakarinen, and A. Soivio. 1995. A dose-response study of thiamine hydrochloride bathing for the prevention of yolk-sac mortality in Baltic salmon fry (M74 syndrome). Page 46 *in* Bengtsson et al. (1995).

Leatherland, J. F. 1993. Field observations on reproductive and developmental dysfunction of introduced and native salmonids from the Great Lakes. Journal of Great Lakes Research 19:737–751.

Leatherland, J. F. 1994. Reflections on the thyroidology of fishes: from molecules to humankind. Guelph Ichthyology Review 2:1–67.

Mac, M. J., C. C. Edsall, and J. G. Seelye. 1985. Survival of lake trout eggs and fry reared in water from the upper Great Lakes. Journal of Great Lakes Research 11:520–529.

Marcquenski, S. V., and S. B. Brown. 1997. Early mortality syndrome in the Great Lakes. Pages 135–153 *in* R. M. Rolland, M. Gilbertson, and R. E. Peterson, editors. Chemically induced alterations in functional development and reproduction in fishes. SETAC (Society of Environmental Toxicology and Chemistry), Pensacola, Florida.

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 indications of thiamin status. Journal of Nutrition 117:1422–1426.

Moccia, R., G. Fox, and A. J. Britton. 1986. A quantitative assessment of thyroid histopathology of herring gull (*Larus argentatus*) from the Great Lakes and a hypothesis on the causal role of environmental contaminants. Journal of Wildlife Diseases 22:60–70.

Neilands, J. B. 1947. Thiaminase in aquatic animals of Nova Scotia. Journal of the Fisheries Research Board of Canada 7:94–99.

Norrgren, L., editor. 1994. Report from the Uppsala workshop on reproduction disturbances in fish. Swedish Environmental Protection Agency Report 4346, Uppsala.

Piper, R. G., and five coauthors. 1982. Fish hatchery management. U.S. Fish and Wildlife Service, Washington, DC.

SAS (Statistical Analysis System). 1994. SAS/STAT guide for personal computers, version 6.10. SAS Institute Inc., Cary, North Carolina.

Simonin, H., J. Skea, H. Dean, and J. Symula. 1990. Summary of reproductive studies of Lake Ontario salmonids. Pages 15–16 *in* M. Mac and M. Gilbertson, editors. Proceedings of the roundtable on contaminant-caused reproductive problems in salmonids. Great Lakes Science Advisory Board, Biological Effects Subcommittee, Windsor, Ontario.