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Thiamine Nutrition, Synthesis, and Retention in Relation to Lake Trout Reproduction in the Great Lakes

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Abstract.—Juvenile and adult lake trout *Salvelinus namaycush* that were fed semipurified, thiamine-deficient diets or alewives *Alosa pseudoharengus* containing thiaminase, a thiamine-destroying enzyme, showed no overt symptoms of thiamine deficiency. Growth rates and ovulation rates were similar among all treatments. However, liver thiamine pyrophosphate (TPP), a biochemical indicator of impending thiamine deficiency, in juvenile lake trout fed thiamine-deficient diets was reduced to 35 pmol/g compared with 59 pmol/g in control groups. Blood TPP in adult female lake trout fed alewives was one-third of that in controls fed a commercial diet. Adult lake trout from Lake Michigan had blood TPP levels similar to those of fish fed the alewife diet in the laboratory. Lake Superior lake trout had TPP levels similar to those of fish fed the control diet in the laboratory. Thiamine synthesis occurred in the intestine of lake trout. At least 81% of thiamine in the posterior intestine was synthesized, presumably by bacteria, when a ^{14}C-labeled thiamine diet was force-fed to lake trout. Thiamine had a long retention time in the lake trout: at 27 weeks after fish were injected with radioactive thiamine, blood cells retained 11% of the radioactivity that was present at 2 d and liver tissue retained 34% of the 2-d level. Lack of self-sustaining lake trout reproduction by Lake Michigan fish may be related to their lower blood thiamine levels. Thiamine deficiency may cause early mortality syndrome, which is common in Lake Michigan but not Lake Superior fish with higher blood thiamine levels.

Forty years after their demise throughout the Great Lakes, self-sustaining lake trout *Salvelinus namaycush* populations have been restored only in Lake Superior. This is in spite of massive stocking of lake trout and control of the sea lamprey *Petromyzon marinus* (Eshenroder et al. 1995; Hansen et al. 1995; Holey et al. 1995). Also during the past 40–50 years, the primary forage species of lake trout in the Great Lakes has shifted from lake herring *Coregonus artedi* and other coregonids to alewives *Alosa pseudoharengus* and smelt *Osmerus mordax* (Berst and Spangler 1972; Lawrie and Rahrer 1972; Wells and McLain 1972). Smelt and alewives have become important components of lake trout diets in the upper Great Lakes (Jude et al. 1987; Diana 1990; Miller and Holey 1992; Conner et al. 1993).

Because smelt and alewives contain thiaminase, a thiamine-destroying enzyme (Gnaedinger and Krzeczkowski 1966), lake trout that consume those species may develop a thiamine deficiency. Such a nutritional deficiency may result in subnormal reproductive success because thiamine is an essential vitamin for fish (Halver 1989). When raw fish containing thiaminase were fed to fish, symptoms of thiamine deficiency were found (Wolf 1942; Harrington 1954). If lake trout in the Great Lakes are suffering from a thiamine deficiency caused by consumption of smelt or alewives, their reproductive success might be impaired. That impairment might manifest itself as early mortality syndrome (EMS) of lake trout larvae (Fitzsimons 1995; Fisher et al. 1996).

Thiamine, a water-soluble vitamin, has a high turnover rate, and deficiency symptoms are rapid and severe in mammals (Gubler 1991). In contrast, the development time for overt symptoms in fish is relatively long (Halver 1957; Coble 1965; Morito et al. 1986; Masumoto et al. 1987; Morris and Davies 1995; Morris et al. 1995). Two possible causes for this prolonged time for development of deficiency symptoms may be that fish can obtain thiamine from a nondietary source or that thiamine is retained by fish for a long period of time.

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The objectives of this study were to (1) determine the effect of thiamine-deficient and thiaminase-containing diets on lake trout reproduction, (2) assess the thiamine nutritional status of adult lake trout in Lakes Superior and Michigan, (3) determine if thiamine is synthesized in the gastrointestinal tract of lake trout, and (4) determine the relative retention time of thiamine in lake trout.

Methods

Feeding Experiments

Three laboratory feeding experiments were conducted. In experiments 1 and 2, adult and juvenile lake trout, respectively, were fed semipurified diets containing different levels of thiamine. In experiment 3, adult lake trout were fed alewives containing thiaminase or a commercial trout diet.

Fish in experiments 1 and 3 were held in circular tanks, 1.8 m in diameter and 90 cm deep, with a volume of about 2,250 L. The tanks were continuously supplied with aerated well water at a constant temperature of 11°C. Water flow of approximately 20 L/min per tank maintained dissolved oxygen concentrations at approximately 8 mg/L. A 60-W fluorescent tube and a 40-W incandescent bulb were suspended above a 50 × 50 cm screened opening in the plywood cover of each tank. Photoperiod was synchronized weekly with the local photoperiod at 45° N latitude. Sunrise and sunsets of 0.5 h were simulated by lighting the incandescent bulb only.

Fish in experiment 2 were held in six covered rectangular tanks, 60 × 50 × 50 cm deep, holding approximately 150 L of water. The flow rate of the 11°C water was approximately 5 L/min. A hole of approximately 10 cm in diameter allowed feeding and limited light penetration; the photoperiod was adjusted weekly to local conditions.

In experiment 1, 60 adult (6 years old) female lake trout broodstock that had previously spawned once in the hatchery were obtained in January 1987 from the Crystal Springs Hatchery, Minnesota Department of Natural Resources. Fish were about 60 cm long and weight averaged 2.1 kg. Ten fish were randomly assigned to each of the six circular tanks. All fish were fed a commercial trout grow-out diet (Glencoe Mills, Glencoe, Minnesota) for 3 months. During that time, food consumption returned from a much reduced rate to the rate at the hatchery before transportation, approximately 0.5% of body weight per day. Semipurified diets containing approximately 0, 1, and 40 mg/kg thiamine were then randomly assigned, two tanks for each diet. Fish were fed to satiation twice daily on weekdays and once daily on weekends. Experiment 1 was conducted for 16 months, from April 1987 to August 1988.

Three attempts were made from mid-November to early December 1987 to manually strip eggs from all fish that had ovulated. Each time a fish ovulated, blood was taken for determination of thiamine nutritional status by measurement of thiamine pyrophosphate (TPP). Length and weight were measured on all fish on the third attempt at stripping on 3 December 1987. On 10 August 1988, an accident occurred in the water supply system that killed about two-thirds of the experimental fish, equally distributed among treatments. Length, weight, and weight of ovaries of the fish that died were measured and liver samples were taken. Ovary weight was used to compute gonadosomatic index as an indicator of reproductive development.

In experiment 2, 90 juvenile lake trout, with an average weight of 387 g, were obtained from the St. Paul Metro Hatchery, Minnesota Department of Natural Resources, and were randomly assigned to the six experimental tanks. All fish were individually tagged and fed the commercial trout diet for 2 weeks and then the semipurified 40 mg/kg thiamine diet for about 1 week. The same semipurified diets used in experiment 1 were then randomly assigned to the tanks. Feeding procedures were the same as in experiment 1.

The experiment ended after approximately 5.5 months when the accident in the water supply system caused the death of all of the fish. Food consumption rate was measured each day during the experimental period. Liver samples were taken from the dead fish at the termination of the experiment.

In experiment 3, Lake Michigan alewives or a commercial trout diet were fed to adult female lake trout. Frozen alewives, purchased from Schilling Fisheries (Ocono, Wisconsin), contained no detectable thiamine. Thiaminase activity, expressed as the amount of thiamine destroyed per gram of fish per minute, was approximately 118 pmol $\cdot$ g^{-1} $\cdot$ min^{-1}.

Thirty-two adult female lake trout, average weight 3.2 kg, from the same source as in experiment 1 were randomly distributed into four experimental tanks in January 1989. They were fed the commercial trout diet for 3 weeks during acclimation to the laboratory. These fish had previously spawned twice at the hatchery.

The experiment was begun on 8 February 1989, when fish in two tanks were fed thawed alewives and fish in the other two tanks continued to receive the commercial diet. All fish were fed to satiation twice daily on weekdays and once daily on week-

TABLE 1.—Composition of the semipurified diets used in feeding experiments 1 and 2. Thiamine was added at the rate of 0, 1, or 40 mg/kg to create the three experimental diets.

Ingredient	Amount (% by weight)
Vitamin-free casein	42
Dextrin	10
Dextrose	7
α-Cellulose	10
Bernhart-Tomarelli Salt mix	5
Herring oil	12
Gelatin	10
Choline Cl	1
Vitamin pre-mix without thiamine[a]	3
Thiamine	0, 0.0001, 0.004
DL-Methionine	0.5
Arginine (free base)	1
L-Tryptophan	0.04

[a] The vitamin premix provided the following vitamin content in 1 kg of feed: vitamin A palmitate, 10,000 IU; vitamin D3, 4,000 IU; vitamin E acetate, 200 IU; vitamin K, 50 mg; riboflavin, 50 mg; biotin, 0.5 mg; folic acid, 20 mg; vitamin B12 crystal, 0.2 mg; niacin, 300 mg; pyridoxine HCl, 40 mg; inositol, 500 mg; ascorbic acid, 500 mg; D-calcium pantothenate, 100 g.

ends. The experiment was conducted for about 21 months, from 8 February 1989 to 12 November 1990. Length and weight were determined and blood samples were taken at the beginning of the experiment and after 5, 10, 14, 18, and 21 months.

A "thiamine-free," casein-based, semipurified diet was made in the laboratory (Table 1). This formulation is that of Poston (1976) with minor modifications by Masumoto et al. (1987) and Morito et al. (1986). Thiamine was added to produce diets with three different thiamine (dry weight) levels: 0, 1, and 40 mg/kg. Because "thiamine-free" ingredients contained trace amounts of thiamine, the actual thiamine contents of the three diets were 0.17, 1.3, and 40 mg/kg. The 1 mg/kg thiamine diet is the recommended minimum needed by salmonids to avoid deficiency symptoms (NRC 1981; Morito et al. 1986). The 40 mg/kg level was chosen because commercial grow-out diets usually contain 20 mg/kg thiamine and manufacturers sometimes double the grow-out vitamin content in broodstock feeds.

All ingredients were purchased from the United States Biochemicals Corp. (Cleveland, Ohio) except herring oil, which was supplied by Glencoe Mills. Animal gelatin, dissolved in hot water, was used as a binder to provide a diet with a soft, rubbery texture with 50% water content. The feed was frozen at −20°C until use.

Thiamine Nutritional Status of Wild Lake Trout

Adult lake trout blood samples for determination of TPP were taken in the vicinity of Saugatuck, Michigan, for Lake Michigan and Bayfield, Wisconsin, for Lake Superior. Reproduction of lake trout in the Saugatuck area was thought to be nonexistent, whereas naturally reproduced lake trout were common in the Bayfield area (Hansen et al. 1995; Holey et al. 1995).

Fish were collected on five dates spanning approximately 14 months. Actual sampling dates for Lake Michigan were 27 September 1987 (prespawning), 2 November 1987 (spawning), 19 May 1988 (spring), 11 September 1988 (prespawning), and 3 November 1988 (spawning). For Lake Superior, sampling dates were 20 September 1987 (prespawning), 22 October 1987 (spawning), 9 May 1988 (spring), 15 September 1988 (prespawning), and 16 October 1988 (spawning).

Fish were captured in gill nets from the Saugatuck area by the U.S. Fish and Wildlife Service. For the spring sampling in 1988, fish were captured by angling. Samples obtained from the Bayfield area were gillnetted by the Red Cliff Band of Lake Superior Chippewa Indians of Wisconsin or the Wisconsin Department of Natural Resources.

Only fish still alive when pulled from the water were used for blood collection. However, because of the multitude of activities on the research vessels, blood could not always be drawn from fish immediately upon capture. A comparison of the stability of TPP levels in blood taken at 0, 20, 40, and 60 min after death indicated no significant differences. Thus, blood samples taken within 1 h of a fish's death adequately represented the TPP levels at the time of death. Blood samples were immediately placed on dry ice for transportation to the laboratory.

Thiamine Synthesis Experiment

Radioactive thiamine as a percentage of total thiamine was used in this experiment to determine the presence or absence of thiamine synthesis in the gastrointestinal tract of lake trout. This approach was based on the principle that a radioactive isotope behaves in the same manner as its nonradioactive counterpart. When radioactive thiamine was fed to fish, ^{14}C thiamine should behave identically to ^{12}C thiamine in the gastrointestinal tract. Radioactive thiamine as a percentage of total thiamine would remain constant as feed passed through the gastrointestinal

tract. The percentage would decrease if a nondietary source of thiamine, such as thiamine synthesis, was present. Thus, the ratios of radioactive thiamine to total thiamine in the contents of the stomach and the anterior and posterior intestine were used to confirm the presence or absence of a nondietary source of thiamine in the gastrointestinal tract.

To conduct this experiment, a feed containing radioactive thiamine was prepared. Thiazole-2-^{14}C-labeled thiamine hydrochloride, in units of 50 μCi and with a specific activity of 24.2 mCi/mmol, was purchased from Amersham Corp. (Arlington Heights, Illinois). A stock solution was prepared by dissolving one 50-μCi unit into 5 mL of 0.1 N HCl. Approximately 1.0 mL of the stock solution was mixed with 1 kg (dry weight) of thiamine-free ingredients to make about 2 kg of moist gelatin feed as described above for the feeding experiments.

The amount of radioactive thiamine (micrograms per kilogram) in the feed and gastrointestinal contents was calculated as:

$$^{14}\text{C thiamine} = [(\text{sample CPM} - \text{blank CPM}) \times \text{MW} \times 1{,}000]/[\text{SW} \times \text{QC efficiency} \times 62.4 \times 2.22 \times 10^6)$$

where CPM is counts per minute, MW is molecular weight, SW is sample weight, QC efficiency is quenching and counting efficiency (95.4%), 62.4 is the theoretical maximum specific radioactivity, and 2.22×10^6 represents disintegrations per minute (dpm) per microcurie. Theoretical maximum specific radioactivity was used to calculate the thiamine content that is 100% radioactive with ^{14}C atoms at the 2-position of the thiazole ring. The radioactive thiamine used contained only 38.78% radioactive thiamine. Quenching and counting efficiency was determined by using a toluene standard spike combined with a factory-sealed ^{14}C standard.

The radioactivity in the feed was confirmed in random samples as 11,799 (SE = 549) dpm/g of feed. This is equivalent to a thiamine supplement of 74.2 (SE = 3.5) μg/kg total thiamine, of which 28.8 μg/kg was ^{14}C thiamine. The trace amount of thiamine that existed in the thiamine-deficient diet was 170 μg/kg (dry weight) and the feed from which radioactivity was measured had a 50% water content; thus, the total thiamine in the feed was approximately 160 μg/kg (wet weight), with ^{14}C thiamine being 18% of the total thiamine. Supplemental thiamine was intentionally low so that the feed would be classified as thiamine-deficient (NRC 1981) yet have sufficient thiamine to be measurable. Wet weight was used because the total thiamine content and radioactive thiamine were analyzed on a wet weight basis.

The fish used in this experiment were from the control groups in feeding experiment 3. The fish were held in a circular tank as described above for feeding experiments 1 and 3, except that a screen was placed about 15 cm from the bottom to minimize the potential for coprophagy by the fish. Before the experiment, fish were fed the thiamine-deficient, semipurified diet for 3 months.

After acclimation to the semipurified diet, 12 fish were starved for 3 d, then anesthetized with tricaine methanesulfate (MS-222), and force-fed approximately 20 g of the radioactive diet per fish with a caulk gun. After 24 h, all fish were killed with an overdose of the anesthetic and dissected. The gastrointestinal tract was removed and separated into three parts: the stomach, the anterior half of the intestine, and the posterior half of the intestine. Approximately 2 g of stomach contents and all of the anterior and posterior intestinal contents were taken. These samples were then divided into two parts: about 0.2 g from each gastrointestinal tract section was placed in tared liquid scintillation vials for radioactive analysis, and the remaining contents were analyzed for total thiamine by column exchange.

To determine the presence and amount of nondietary thiamine, the following equations were used:

$$T_1 = (1 - R_a/R_s) \times 100$$

$$T_2 = (1 - R_p/R_s) \times 100$$

where T_1 and T_2 are the percentages of nondietary thiamine in the anterior and posterior intestine, respectively; R_a is the ratio of radioactive thiamine to total thiamine in the contents of the anterior intestine; R_s is the ratio of radioactive thiamine to total thiamine in the stomach contents (diet); and R_p is the ratio of radioactive thiamine to total thiamine in the contents of the posterior intestine. To distinguish the percentage of nondietary thiamine in the posterior intestine that originated in the anterior intestine, the following formulas were used:

$$T_3 = (1 - R_p/R_a) \times 100$$

$$T_4 = (R_p/R_a - R_p/R_s) \times 100$$

where T_3 is the percentage of nondietary thiamine of posterior origin and T_4 is the percentage of nondietary thiamine of nonposterior origin.

To determine tissue uptake of radioactive thiamine from the fish that were force-fed the ^{14}C-labeled thiamine, approximately 0.2 g of liver, stomach, pyloric ceca, and anterior and posterior intestine were washed and placed in tared liquid scintillation vials. The tissues were then digested with 1.5 mL of Soluene (Packard Instruments Co., Meriden, Connecticut) for 48 h in a 55°C water bath. Scintillation fluid (15 mL of Ecocint A, National Diagnostics, Manville, New Jersey) was added and radioactivity was measured with a Beckman LS-1000 liquid scintillation spectrometer (Beckman Instruments, Inc., Schaumburg, Illinois).

Thiamine Retention Experiment

Fish from the same source and holding conditions as in the thiamine synthesis experiment were used to determine the length of time that thiamine was retained in various fish tissues. Fish were fed the semipurified, thiamine-deficient diet for 3 months before and for the 26-week experimental period.

Units of thiazole-2-^{14}C thiamine were dissolved in a 0.9% NaCl solution. A dose of approximately 1 μCi/kg of fish was injected into the caudal artery after the fish were anesthetized with MS-222. Blood samples were taken from the caudal artery 48 h after injection with a 10-mL heparinized Vacutainer tube. The blood was then centrifuged at 6,500 g for 10 min. The supernatant was discarded and 200 μL of packed cells was pipetted into a scintillation vial. The elapsed time of 48 h ensured that the injected thiamine had sufficient time to be taken into blood cells. This was confirmed by samples of plasma taken 48 h after injection in which radioactivity was only slightly above background and negligible compared with that in blood cells.

Liver biopsy samples were taken with a 14-gauge, 11.4-cm Monoject Actuated Biopsy Cutting Needle (Sherwood Medical, St. Louis, Missouri). After a fish was anesthetized, a 4-mm incision in the integument was made posterior to the base of the pelvic fins. About 20–50 mg of liver was obtained from each fish and placed in a tared liquid scintillation vial. Multiple biopsy sections were needed to obtain the desired amount of sample material. Occasionally, inadequate amounts were obtained so that data were not available from all fish at all sampling dates. Oxytetracycline was injected into each fish to prevent possible infection of the incision. The sampling of packed cells and liver tissue was repeated at 42, 109, and 171 d after injection.

On day 42 after the injection, two fish were killed to determine the distribution of radioactivity in various organs. On day 189, the experiment was terminated. Radioactivity was determined in about 0.2 g of the muscle, liver, stomach, heart, kidney, spleen, intestine, and ovarian tissue of the eight remaining fish.

Blood Sampling Procedures and Chemical Analysis

Except as noted otherwise, blood samples were drawn in the following manner in the laboratory and field. Fish were first anesthetized with MS-222. Blood (2–3 mL) was drawn from the caudal artery into a 10-mL heparinized Vacutainer tube with a 22-gauge needle. The blood samples were placed on ice before transfer to a freezer and then stored at −20°C until analysis.

Blood and liver TPP, the active coenzyme form of thiamine, was used as an indicator of thiamine nutritional status. TPP levels were determined by high-pressure liquid chromatography (HPLC). The procedures of Baines (1985) were followed except that whole blood was used instead of erythrocytes. The preparation of liver tissue was according to Masumoto et al. (1987) except that TPP was extracted into methanol instead of trichloroacetic acid, and then Baines' (1985) method was followed.

A recovery experiment with a pooled lake trout blood sample was conducted to measure the percentage of standard TPP extracted and measured by the HPLC procedure. After the first centrifugation for removal of debris, the pooled sample was divided into three tubes of 1.8 mL each. One tube received no TPP spike, one was spiked with 0.2 mL of 1 pmol/mL TPP standard solution to achieve an increase in TPP content of 100 pmol/mL, and one was spiked with 0.2 mL of 4 pmol/mL TPP standard solution to achieve an increase in TPP content of 400 pmol/mL. The recovery rates were approximately 92 and 94% for the 100 and 400 pmol/mL spikes, respectively.

The stability of TPP during storage was tested. Approximately 60 mL of blood was taken from a group of juvenile lake trout. The pooled blood was then divided into four groups. One group was analyzed for TPP immediately and the others were analyzed after 1, 2, or 4 weeks of storage at −20°C. No significant difference in TPP was found among the four groups.

Because blood could not always be frozen immediately, a comparison was made of the effects on TPP content of different times elapsed before freezing. The TPP content of blood frozen 3 h after drawing was 96% of that of blood frozen immediately

TABLE 2.—Mean weight, number of ovulated lake trout, and blood thiamine pyrophosphate (TPP) levels for lake trout fed for 8 months on semipurified diets containing three different thiamine levels in experiment 1. Values shown in parentheses are SEs.

Dietary thiamine (mg/kg)	Number of fish	Number ovulated	Initial weight (kg)	Weight gained (kg)	Blood TPP (pmol/mL)
0	20	11	2.2 (0.13)	0.25	63 (6)
1	20	10	2.0 (0.11)	0.22	57 (7)
40	17	9	2.1 (0.14)	0.31	73 (7)

after drawing. These differences were not statistically significant. All analyses for total thiamine were conducted by Medallion Laboratories, General Mills, Inc. (Minneapolis, Minnesota).

Statistical Analysis

A randomized block design with multiple group comparisons was used to analyze for differences in blood or liver TPP contents in feeding experiments 1 and 2. A *t*-test was used in experiment 3 to compare blood TPP levels between lake trout fed alewives and those fed the commercial diet at different times. Also, *t*-tests were used in the field surveys to compare blood TPP levels between Lake Michigan and Lake Superior fish.

Paired comparison *t*-tests were used to compare percentages of radioactive thiamine in anterior and posterior intestinal contents with those of stomach contents in the same fish. Samples with total thiamine concentrations below the minimum detectable limit (50 μg/g) were excluded from the statistical analysis. As a result, sample sizes were reduced to six for anterior and five for posterior intestinal contents.

Regression analysis was used to determine the relationship between radioactivity in packed blood cells or in liver and days after thiamine injection. Two-factor analysis of variance was used to compare radioactivity in different organs at different times in the thiamine retention experiment. Unless noted otherwise, significant differences were those with P-values < 0.05.

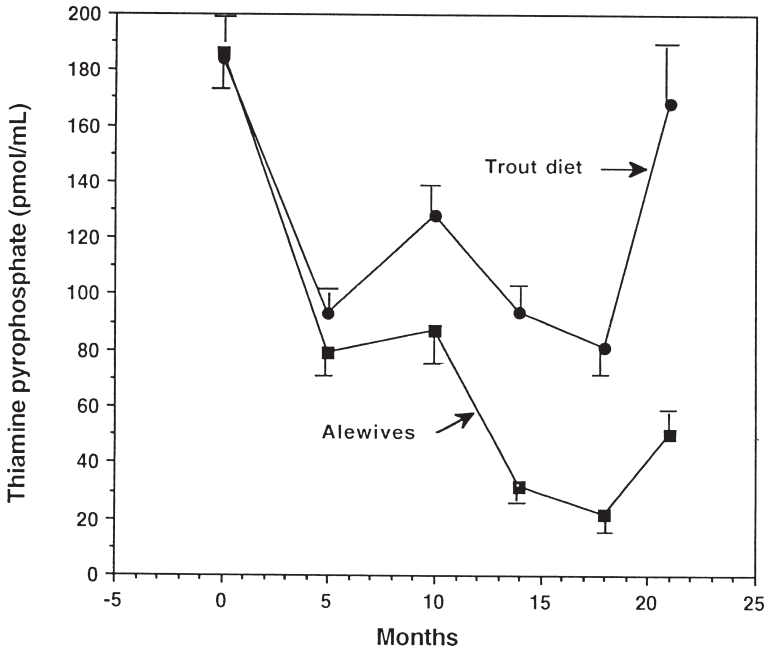

FIGURE 1.—Thiamine pyrophosphate levels in blood of lake trout fed a commercial trout diet or alewives containing thiaminase at various times after the start of experiment 3. Bars shown above or below the mean are SEs.

TABLE 3.—Mean weight, liver thiamine pyrophosphate (TPP) levels, and gonadosomatic index (grams of ovaries per gram of fish × 100) for lake trout fed for 16 months on the semipurified diets in experiment 1. Values shown in parentheses are SEs.

Dietary thiamine (mg/kg)	Number of fish	Weight (kg)	Liver TPP (pmol/g)	Gonadosomatic index
0	14	2.8 (0.29)	204 (22)	5.5 (0.94)
1	14	2.3 (0.27)	150 (24)	4.9 (1.24)
40	12	2.9 (0.28)	191 (33)	5.4 (0.95)

Results

Feeding Experiments

In experiment 1, growth rates were not significantly different among adult lake trout receiving 0, 1, or 40 mg/kg thiamine. Fish in all tanks fully consumed the 0.5% daily ration. However, fish gained little weight during the experiment (Table 2). Three fish that received the 0 mg/kg thiamine diet died from unknown causes.

Many fish ovulated during the spawning season, approximately 8 months after the start of the experiment. The percentage of fish that ovulated was similar among the three diet treatments: 55% for fish fed the 0 mg/kg thiamine diet, 50% for fish fed the 1 mg/kg thiamine diet, and 53% for fish fed the 40 mg/kg thiamine diet (Table 2). The TPP levels in blood at the time of ovulation were not significantly different among fish fed the three diets.

About 70% of the experimental fish died 16 months after the start of the experiment after an accident in the water supply system. At that time, neither weight, gonadosomatic index, nor liver TPP levels differed significantly as a result of diet (Table 3).

In experiment 2, the juvenile lake trout fed diets containing the three different levels of thiamine had similar food consumption and growth rates after 5.5 months (Table 4). Liver TPP levels were significantly different among the experimental groups ($P < 0.001$). Fish fed the 0 mg/kg thiamine diet had the lowest level of liver TPP, whereas those fed the 1 mg/kg thiamine diet had the highest liver TPP concentration.

In experiment 3, essentially all energy consumed went to the production of eggs, because after eggs were stripped at 9 and 21 months, somatic growth ranged from slightly negative to slightly positive with no significant difference among treatments (Table 5). During the 1989 spawning season, 87% of the fish ovulated, and during 1990, 86% ovulated, with no significant difference between fish fed the commercial diet and the alewives.

Fish fed either alewives or the commercial diet had similar blood TPP levels (approximately 185 pmol/mL) at the start of the experiment (Figure 1). After 5 months, blood TPP contents of fish fed alewives began to differ from those of fish fed the commercial diet. These differences became statistically significant ($P < 0.001$) by the 10th month and remained so thereafter.

Thiamine Synthesis Experiment

Mean total and radioactive thiamine concentrations declined between the stomach contents and the contents of the two intestinal segments (Table 6). The thiamine concentration measured in the stomachs of force-fed lake trout was 153 μg/kg, with 20.2% radioactive; this approximated the level that we estimated for the feed, 160 μg/kg, with 18% radioactive. Radioactive thiamine as a percentage of total thiamine declined progressively as food passed

TABLE 4.—Mean gain in weight, food consumption rate, and liver thiamine pyrophosphate (TPP) levels of juvenile lake trout fed semipurified diets for 5.5 months in experiment 2. Values shown in parentheses are SEs.

Dietary thiamine (mg/kg)	Initial weight (g)	Weight gain (g)	Food consumption (grams of fish per week)	TPP levels (pmol/g)
0	389 (23)	160 (16)	49.3 (2.2)	35 (2.8)
1	373 (15)	151 (24)	47.1 (0.9)	72 (6.1)
40	397 (17)	160 (10)	48.6 (1.0)	59 (5.2)

TABLE 5.—Mean weight (kg) and number of ovulated lake trout in experiment 3. Fish were fed either a commercial pelleted trout diet or alewives containing thiaminase, over 21 months, including two spawning cycles. Values shown in parentheses are SEs.

Months after start	Diet type	Number of fish	Number ovulated	Initial weight	Weight after egg stripping
10	Alewives	16	14	2.61 (0.17)	2.68 (0.28)
	Commercial	15	13	2.95 (0.20)	2.79 (0.22)
21	Alewives	14	12	2.83 (0.26)	3.07 (0.28)
	Commercial	14	12	2.80 (0.21)	2.89 (0.20)

from the stomach to the anterior intestine to the posterior intestine, with both anterior and posterior intestinal contents statistically different from the stomach content ($P < 0.001$) but not different from each other (Table 6). The percentages of nondietary thiamine were calculated as 72.8% in the anterior intestine and 81.2% in the posterior intestine, with 30.9% of the latter of posterior origin and 50.3% of nonposterior origin.

Radioactive thiamine was absorbed into the various tissues of the gastrointestinal tract and the liver. Tissue radioactivity in increasing order (on a wet tissue basis) was: stomach, 299 dpm/100 mg (SE = 32.7); posterior intestine, 300 dpm/100 mg (SE = 34.5); anterior intestine, 400 dpm/100 mg (SE = 38.0); pyloric ceca, 524 dpm/100 mg (SE = 32.7); liver, 660 dpm/100 mg (SE = 69.4).

Thiamine Retention Experiment

Radioactive thiamine had a relatively long retention time in both packed cells and liver tissue of lake trout. At 27 weeks, packed blood cells still retained 11% of the radioactivity present at 2 d and liver retained 34% of the 2-d level (Figure 2).

The radioactivity in different tissues changed between samples taken at 6 and 27 weeks, although the data from 6 weeks are somewhat uncertain because of the small sample size (only two fish; Table 7). At 6 weeks, the highest level of radioactivity was in heart tissue, followed by liver, kidney, and ovaries. At 27 weeks, heart tissue continued to have the highest radioactivity, but liver tissue had lower levels than ovarian and kidney tissue. There was a significant difference in radioactivity among tissues at the different times and also a significant interaction

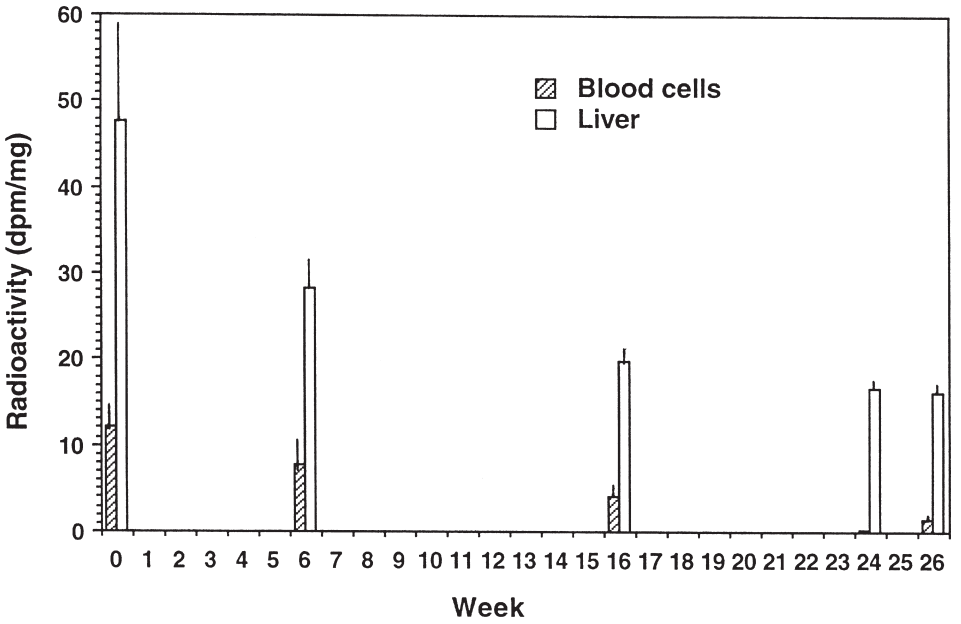

FIGURE 2.—Radioactivity (disintegrations per minute; dpm) in packed blood cells and liver tissue of lake trout at various times after injection of ^{14}C-labeled thiamine. Bars indicate SE values.

TABLE 6.—Total thiamine, radioactive thiamine, and the percentage of radioactive thiamine in the contents of the stomach, anterior intestine, and posterior intestine of lake trout. Fish were force-fed a diet containing ^{14}C-labeled thiamine and samples were taken 24 h later. Values shown in parentheses are SEs.

Contents site	Number of samples	Total thiamine (μg/kg)	Radioactive thiamine (μg/kg)	Percentage radioactive thiamine
Stomach	12	153 (16)	30.0 (4.0)	20.2 (1.8)
Anterior intestine	6	91 (11)	4.8 (0.8)	5.5 (0.9)
Posterior intestine	5	108 (16)	3.7 (0.6)	3.8 (0.7)

between tissue radioactivity and time ($P < 0.01$). Thus, radioactivity was not moving into different tissues uniformly: while radioactivity in some tissues declined, it increased in others.

Thiamine Nutritional Status of Wild Lake Trout

Throughout the 14 months of sampling in the Bayfield area for Lake Superior lake trout and in the Saugatuck area for Lake Michigan lake trout, blood TPP levels were significantly lower ($P < 0.01$) in the Lake Michigan fish (Figure 3). TPP levels of Lake Superior fish were usually at least two times those of Lake Michigan fish.

Discussion

Feeding Experiments

Thiamine, a water-soluble vitamin, generally has a high turnover rate and is not thought to be stored in large amounts or for any substantial period of time in any tissue, at least in mammals (Gubler 1991). Insufficient intake of thiamine results in rapid development of clinical symptoms of deficiency (Gubler 1991). This deficiency has been reported to cause beriberi disease in humans (Shimozono and Katsura 1965), Chastek's paralysis in foxes (Green and Evans 1940), bracken staggers in horses (Evans 1975), and cerebrocortical necrosis in cattle and sheep (Edwin et al. 1979).

In fish, a dietary thiamine requirement has been demonstrated in many species, and typical symptoms of deficiency include anorexia, muscle atrophy, convulsions, loss of equilibrium, edema, hyperexcitability, and poor growth (NRC 1983; Halver 1989). In contrast to mammals, overt thiamine deficiency symptoms in fish take a relatively long time to develop. Various studies have reported development times ranging from 8 to 14 weeks for smaller fish (Halver 1957; Morito et al. 1986; Morris and Davies 1995; Morris et al. 1995) and up to 30 weeks with no symptoms for larger fish (Coble 1965).

In the present study, we observed none of the overt symptoms of thiamine deficiency described above. Juvenile and adult fish fed thiamine-deficient or thiaminase-containing diets for 5.5, 16, or 21 months in three experiments showed no differences in behavior, food consumption rate, growth, or ovulation rate. However, biochemical indicators of thiamine deficiency were detected in two experiments. Juvenile fish in experiment 2 had reduced liver TPP levels after 5.5 months of consuming a semipurified, thiamine-deficient diet of 0.17 mg/kg thiamine. In experiment 3, adult fish fed a thiaminase-containing diet of alewives had lower blood TPP concentrations by 10 months into the experiment than fish fed a commercial diet. Blood and liver TPP is reported to be a sensitive indicator of the potential onset of thiamine deficiency in trout (Masumoto et al. 1987), yet overt symptoms were not observed.

It is uncertain why the adult lake trout did not exhibit overt symptoms of thiamine deficiency. In experiment 1, fish fed a semipurified, thiamine-deficient diet did not even show reduced blood TPP after 8 months or reduced liver TPP after 16 months. As indicated by our thiamine synthesis and thiamine retention experiments, a nondietary source of thiamine and a long retention time of thiamine in fish tissues may be two factors that contributed to this prolonged time for development of symptoms of thiamine deficiency.

TABLE 7.—Radioactivity (disintegrations per minute per milligram) in different tissues of lake trout at 6 and 27 weeks after injection of ^{14}C-labeled thiamine. Values shown in parentheses are SEs.

Tissue	Week 6	Week 27
Heart	62 (5.1)	36 (5.0)
Liver	36 (6.2)	16 (1.3)
Kidney	21 (10.6)	22 (2.8)
Ovaries	20 (6.2)	25 (6.8)
Spleen		20 (2.0)
Stomach	20 (0.5)	9 (1.2)
Intestine	18 (1.2)	11 (1.1)
Packed cells	8 (1.9)	1 (0.5)
Muscle	5 (0.5)	5 (0.8)

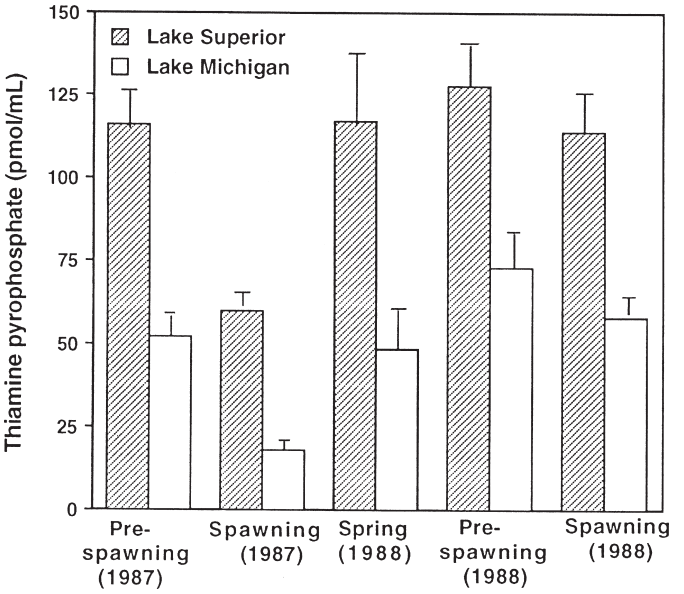

FIGURE 3.—Mean thiamine pyrophosphate levels in blood of lake trout from Lakes Michigan and Superior. See text for actual sampling dates. Bars indicate SE values.

Thiamine Synthesis Experiment

The significant decrease in percentage of radioactive thiamine from stomach contents, anterior intestinal contents, and posterior intestinal contents indicated a significant nondietary source of thiamine that increased the percentage of nonradioactive thiamine in the intestine of lake trout.

Thiamine is not known to be synthesized by animals, only by bacteria, yeast, and higher plants (Gubler 1991). Among mammals, substantial thiamine synthesis occurs in ruminants, but that synthesis is of microbial origin (Breves et al. 1980, 1981). In ruminants, absorption of thiamine occurs in the small intestine (Miller et al. 1986a, 1986b) through a carrier-mediated process (Hoyumpa 1982). In nonruminant mammals, significant synthesis of thiamine may occur in the gastrointestinal tract (Shibata 1950; Wostmann et al. 1962), but that thiamine is generally believed to be unavailable to the host animal without coprophagy (Gubler 1991). Without coprophagy by lake trout, intestinal absorption would have to occur for synthesis to make a contribution to thiamine nutrition. Although we occasionally observed lake trout ingesting feces in feeding experiments 1 and 3, they invariably ejected it immediately, and the screened tank bottom largely prevented consumption of feces in the thiamine synthesis experiment.

Synthesis of thiamine in the lake trout intestines seems plausible because synthesis of vitamin B or vitamin B-producing bacteria has been found in other fish species. Trepsiene et al. (1977) isolated thiamine-producing bacteria in carp *Cyprinus carpio* intestines. Sugita et al. (1991) isolated vitamin B_{12}-producing bacteria from six freshwater fishes. Limsuwan and Lovell (1981) and Lovell and Limsuwan (1982) reported intestinal synthesis and absorption of vitamin B_{12} in channel catfish *Ictalurus punctatus* and *Tilapia*.

The actual level of thiamine in the anterior intestine of the lake trout in our synthesis study was very low (0.0048 mg/kg) compared with the minimum requirement in feed (1 mg/kg) recommended by the National Research Council (NRC 1981). However, we do not know if synthesized thiamine may have been absorbed rapidly by the intestine and thus may have contributed significantly to thiamine nutrition, despite its low concentration in the intestinal contents. With nondietary thiamine content in the posterior intestine as high as 81% of total thiamine, the contribution to thiamine nutrition of thiamine synthesized in the gastrointestinal tract may be quite significant.

To our knowledge, our method for determining thiamine synthesis has not previously been used. Measurement of nutrient absorption in the intestine was complicated by a potential new source of thiamine, microbial synthesis. The ratio

of radioactive thiamine to total thiamine enables detection of the addition of nondietary thiamine; however, our method cannot measure absorption rate. When synthesis is present, use of a nondigestible marker such as chromic oxide still would not permit determination of absorption because of the addition of nondietary thiamine. The combination of the radioactivity and chromic oxide would reveal the absorption rate of the dietary thiamine alone by measuring radioactive thiamine against the indigestible marker. However, it would not address the absorption rate of nondietary thiamine because the total quantity of nondietary thiamine remains unknown.

Our measurement of radioactive thiamine in contents of the gastrointestinal tract assumed that all measured radioactivity resulted from intact ^{14}C-labeled thiamine, but in reality, measured radioactivity could also have resulted from degraded thiamine. Radioactive and nonradioactive thiamine degrade at the same rate. Measurement of radioactivity in degraded thiamine would give the appearance of a higher percentage of radioactive thiamine in the intestinal contents and lead to the conclusion that a smaller increase in nondietary thiamine had occurred than was the case. Thus, our measurement is conservative.

Although thiamine synthesis seemed to occur in the gastrointestinal tract of the lake trout, it is difficult to ascertain what role that synthesis might play in preventing thiamine deficiency in field situations. If a thiamine deficiency in female spawners causes a deficiency in eggs that results in EMS, then thiamine synthesis is not sufficient to overcome that deficiency. However, if the deficiency is caused by the consumption of thiaminase-containing prey, as is assumed, the thiaminase may remain active in the intestine and thus negate any synthesis that might occur. In feeding experiment 1, no evidence of thiamine deficiency was found in lake trout fed a thiamine-deficient diet for 16 months, but in experiment 3, fish had reduced blood TPP levels after 10 months of feeding on thiaminase-containing alewives. This suggests that the presence of thiaminase had a greater effect on the development of thiamine deficiency than the absence of thiamine in the diet.

Thiamine Retention Experiment

The long retention time of thiamine by lake trout may also contribute to the prolonged time for development of thiamine deficiency symptoms in fish in the feeding experiments. At 27 weeks after injection, radioactivity in blood cells was still 16% of radioactivity at 2 d and radioactivity in liver was 34% of the 2-d levels. However, while those tissues were losing thiamine, it was increasing in other tissues, particularly kidney and ovaries.

Very few thiamine turnover or thiamine retention studies have been done with mammals, and we are aware of none with fish. Trebukhina et al. (1985) injected ^{14}C thiamine into mice fed a thiamine-deficient diet and reported the rate at which thiamine was incorporated into various tissues. The tissues with high turnover rates were liver, kidney, heart, stomach, spleen, and brain. In the present study, we measured the retention rate, or the rate at which radioactive thiamine was depleted, as opposed to the rate at which radioactive thiamine was incorporated. We found high radioactivity in the same tissues in which Trebukhina et al. (1985) found it in mice.

Thiamine Nutritional Status of Wild Lake Trout

The thiamine nutritional status of fish collected from Lake Michigan resembled that of lake trout fed the alewife diet for 10 months in feeding experiment 3. Blood TPP levels of fish from Lake Michigan ranged from 18 to 73 pmol/mL for samples taken over 14 months (Figure 3). The TPP levels in lake trout fed the thiaminase-containing diet ranged from 22 to 87 pmol/mL for the period between 10 months, when TPP levels first differentiated from controls, and 21 months, when the experiment was terminated (Figure 1). The blood TPP levels in Lake Superior lake trout, which ranged from 60 to 128 pmol/mL, were similar to those of control fish in experiment 3, which ranged from 81 to 168 pmol/mL.

The difference in blood TPP levels between lake trout from Lakes Michigan and Superior may be caused by a combination of diets composed of thiaminase-containing species and the amount of thiaminase activity in those prey species. In the vicinity of Bayfield, Lake Superior, thiaminase-containing smelt accounted for 66% of lake trout diets in 1987 (Conner et al. 1993). In Lake Michigan, the diet of nearshore lake trout during 1984–1988 consisted of 81% thiaminase-containing alewives (Miller and Holey 1992). In lake trout sampled in 1986 from Saugatuck, Lake Michigan, alewives constituted 82% and smelt constituted 2% of lake trout stomach contents (R. F. Elliot, U.S. Fish and Wildlife Service, unpublished data). The remaining 16% of the diet consisted of bloaters *Coregonus hoyi* and yel-

low perch *Perca flavescens*, which are not known to contain thiaminase. Whether the higher percentage of thiaminase-containing forage fish consumed by Lake Michigan lake trout was sufficient to cause their lower thiamine nutritional status is uncertain. Also, it is difficult to assess whether differences in thiaminase activity between alewives and smelt caused the differences in blood TPP levels between lake trout from the two lakes. Ji and Adelman (1998, this volume) found that thiaminase activities of these two species were highly variable depending on species, sampling location, and time, with a significant interaction among these variables.

The lower TPP levels in Lake Michigan lake trout compared with Lake Superior fish suggest a possible link between thiamine nutritional status and reproductive success, including the occurrence of EMS (Fitzsimons 1995; Fisher et al. 1996). Self-sustaining populations of lake trout have been reestablished in most of Lake Superior (Hansen et al. 1995), whereas self-sustaining reproduction of lake trout in Lake Michigan has been nonexistent (Holey et al. 1995). Furthermore, EMS has been observed in lake trout from Lake Michigan but not Lake Superior (Fitzsimons 1995). However, the level of TPP activity in blood below which females produce larvae having EMS remains to be established.

At the time the present study was undertaken, thiamine deficiency had not been implicated in EMS of lake trout sac fry (Fitzsimons 1995; Fisher et al. 1996). From our laboratory experiments and field observations, it appears that adult lake trout can ovulate and spawn successfully when consuming thiamine-deficient or thiaminase-containing diets. However, sufficient thiamine for ovulation and spawning may not be enough to prevent EMS in sac fry, particularly because lake trout in Lake Michigan feed on thiaminase-containing species for most of their lives.

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