Journal of Aquatic Animal Health 17:48–58, 2005

DOI: 10.1577/H04-003.1

[Article]

The Effect of Thiamine Injection on Upstream Migration, Survival, and Thiamine Status of Putative Thiamine-Deficient Coho Salmon

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Abstract.—A diet containing a high proportion of alewives *Alosa pseudoharengus* results in a thiamine deficiency that has been associated with high larval salmonid mortality, known as early mortality syndrome (EMS), but relatively little is known about the effects of the deficiency on adults. Using thiamine injection (50 mg thiamine/kg body weight) of ascending adult female coho salmon *Oncorhynchus kisutch* on the Platte River, Michigan, we investigated the effects of thiamine supplementation on migration, adult survival, and thiamine status. The thiamine concentrations of eggs, muscle (red and white), spleen, kidney (head and trunk), and liver and the transketolase activity of the liver, head kidney, and trunk kidney of fish injected with thiamine dissolved in physiological saline (PST) or physiological saline only (PS) were compared with those of uninjected fish. The injection did not affect the number of fish making the 15-km upstream migration to a collection weir but did affect survival once fish reached the upstream weir, where survival of PST-injected fish was almost twice that of controls. The egg and liver thiamine concentrations in PS fish sampled after their upstream migration were significantly lower than those of uninjected fish collected at the downstream weir, but the white muscle thiamine concentration did not differ between the two groups. At the upper weir, thiamine levels in the liver, spleen, head kidney, and trunk kidney of PS fish were indistinguishable from those of uninjected fish (called "wigglers") suffering from a severe deficiency and exhibiting reduced equilibrium, a stage that precedes total loss of equilibrium and death. For PST fish collected at the upstream weir, total thiamine levels in all tissues were significantly elevated over those of PS fish. Based on the limited number of tissues examined, thiamine status was indicated better by tissue thiamine concentration than by transketolase activity. The adult injection method we used appears to be a more effective means of increasing egg thiamine levels than immersion of eggs in a thiamine solution.

A diet-related thiamine deficiency results in early mortality syndrome (EMS) in the larval stages of Lake Michigan coho salmon *Oncorhynchus kisutch* (Fitzsimons et al. 1999; Marcquenski and Brown 1997; Hornung et al. 1998; McDonald et al. 1998). Early mortality syndrome is a collection of behavioral and morphological signs observed in affected larvae that include loss of equilibrium, alternating hyperexcitability and lethargy, and re-

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Received January 5, 2004; accepted November 30, 2004
Published online May 10, 2005

duced yolk sac utilization, which eventually result in death. Symptoms resembling those of EMS have been seen in larval coho salmon since 1966 (Fitzsimons et al. 1999).

Despite the early evidence of a thiamine deficiency in larval stages of coho salmon, there was no indication that adult parents of larvae developing EMS were suffering effects of a thiamine deficiency until 1996. At that time, Michigan Department of Natural Resources hatchery personnel observed unusual behavior in migratory adult coho salmon at a collection weir on the Platte River, a tributary to northeastern Lake Michigan. The behavior, termed wiggling, was clearly distinguishable from normal behavior and was characterized by a loss of equilibrium wherein affected fish had difficulty remaining upright, literally rolling onto their sides when changing direction during swimming. Suspicion was raised as to the possible involvement of thiamine because of the similarity of signs with those of a thiamine deficiency affecting adult Baltic salmon *Salmo salar*, in which affected fish showed wiggling and lack of coordination, and in severe cases, death (Amcoff et al. 1998). Accompanying these adult effects was a larval mortality syndrome similar to EMS, known as M74 (Amcoff et al. 1998, 1999).

Samples collected from wiggling and distressed adult Lake Michigan coho salmon in 1999 confirmed that adults were indeed suffering from a severe thiamine deficiency when compared with normally behaving fish from other locations (Brown et al. 2005, this issue). This was also a year when over 90% of Platte River coho salmon females had progeny that displayed EMS, which resulted in 100% mortality (Wolgamood et al. 2005, this issue).

Given the variety of clinical signs associated with the thiamine deficiency in adult coho salmon up until 2000, which varied from altered swimming behavior to mortality, the possibility existed that their ability to undertake an upstream spawning migration may be adversely affected. Normally, spawning places a heavy requirement on body energy stores (Brett 1995) that can vary with the velocity and amount of turbulence encountered during the instream migration (Hinch and Rand 1998). Utilization of body energy stores to support metabolism is highly regulated by thiamine. Thiamine is involved in generating ATP equivalents (NADH, NADPH) through three thiamine-dependent enzymes: transketolase (enzyme number 2.2.1.1; IUBMB 1992) of the pentose phosphate shunt, pyruvate dehydrogenase (1.2.4.1) in the glycolytic pathway, and α-ketoglutarate dehydrogenase (1.2.4.2) in the citric acid cycle. All three enzymes are essential for cell metabolism and require thiamine in its phosphorylated form (i.e., thiamine pyrophosphate [TPP]) for their catalytic activity. Given how essential these enzymes are to cellular metabolism, it seems likely that a number of subcellular effects in thiamine-deficient fish may precede the manifestation of obvious clinical signs of a thiamine deficiency (e.g., behavior) and may significantly constrain energy mobilization for migration.

The nervous system is also vulnerable to the effects of a thiamine deficiency. The central nervous system derives most of its energy from the metabolism of glucose (Morito et al. 1986). With thiamine acting as an important co-enzyme in carbohydrate metabolism, utilization of glucose in fish could be reduced by as much as 50–60% when in a thiamine-deficient state, such as occurs in mammals, severely affecting neurological function (Guyton 1981). Adult Baltic salmon displaying overt neurological signs of thiamine deficiency have altered serotonergic and dopaminergic activities (Amcoff et al. 2002). Conditions of low energy and compromised neurological function resulting from a thiamine deficiency could potentially be further exacerbated by effects on the cardiovascular system. In mammalian species, thiamine deficiency reduces cardiac contractility, heart rate, and blood pressure (Cappelli et al. 1990; Onodera et al. 1991), thereby increasing susceptibility to cardiomegaly and congestive heart failure (Caster and Meadows 1980).

Because of its wide range of effects on metabolic, nervous system, and cardiac function, a thiamine deficiency could exert a considerable impact on the upstream migration, depending on the level of the deficiency. Effects could include an inability to migrate upstream, especially through areas with obstructions or fast water; difficulty in initiating and completing spawning; or mortality occurring either en route to or at the spawning grounds.

To test the hypothesis that thiamine status was affecting migratory ability and that fish were in fact affected by a thiamine deficiency, we injected equal numbers of female coho salmon collected near the mouth of the Platte River with either a thiamine solution (50 mg thiamine/kg body weight) or a physiological saline (PS) solution. We then monitored the number of injected fish that were able to migrate to an upstream weir and evaluated their condition (e.g., alive or dead) and thi-

FIGURE 1.—Map showing the location of the Platte River, Michigan.

amine status when reaching the weir. Herein we report the results of our investigation.

Methods

Location.—The Platte River drains an area of approximately 525 km² in northwestern lower Michigan and passes through mostly mixed deciduous hardwoods and conifers (Figure 1). Collection weirs associated with the Platte River State Fish Hatchery are located approximately 1 and 16 km from the river mouth. The reach between the two weirs represents a distance of 15 km and has an average gradient of less than 1%. There are no significant obstructions on the river between the two weirs. Platte Lake is located on this section of river and has an area of 26 km², an average depth of 8.2 m, and a length of 5 km. The river is open to sportfishing from the downstream weir to the upstream weir; the daily catch limit during the spawning period is five fish per angler.

Treatments.—Physiological saline solution (7.5 g of NaCl/L) was prepared with distilled water from the Canadian Center for Inland Waters (Burlington, Ontario) approximately 5 d before use and was kept cold (5°C) until use. No attempt was made either to prepare solutions from sterile ingredients or to maintain them in a sterile condition once prepared. Thiamine hydrochloride (Sigma Chemical Co., Oakville, Ontario) was dissolved into PS to make up a solution of 27.5 mg/L, sufficient to deliver 50 mg of thiamine per kilogram body weight to 2.5–4.5-kg fish in solution volumes from 5 to 9 mL. The pH of the solution was adjusted to between pH 5–6 by use of 10-N NaOH to maintain stability of the thiamine, which degrades at neutral and higher pH (Jansen 1972). Thiamine solutions were made approximately 12 h prior to use. Clean, 10-mL plastic syringes (22 g, Luer-Loc) were filled to a volume of 10 mL with either PS or thiamine dissolved in PS (PST) on the night before use and were kept on ice until the time of use. Any syringes that were not used on the day after being filled were discarded.

Fish collection at the downstream weir.—Coho salmon used to assess the effect of thiamine on upstream migration were captured at the downstream weir on the Platte River, approximately 1 km from the mouth. Fish were collected as they ascended the river over a 4-d period (September 11–14, 2001). Water temperatures in the river at the weir during this period averaged 22.5°C. Only females exhibiting normal behavior were used, and this constituted close to 100% of the fish observed over the 4-d collection period. At the time of collection, oogenesis was complete but fish had not yet ovulated. Fish migrating up the river were diverted by flow augmentation into a side channel and then were crowded into a lifter. The lifter raised the fish up to a sorting table, where females were randomly selected and directed into a water-filled chute connected to an instream holding pen (2 × 1 × 1 m) located approximately 50 m upstream of the weir. Fish were individually removed from the holding pen, one at a time, for processing.

Fish processing.—Fish were hand-netted out of the instream holding pen and weighed (±1 g) in a mesh bag on a calibrated spring balance located in the stream next to the processing table. No anesthesia was used. Fish, still in the mesh bag, were placed on the table, where they were alternately injected with one of the two treatments. Injection was made just anterior of the vent into the abdominal cavity while avoiding any organs. The volume of solution injected into each fish was calculated on the basis of weight: 2 mL of solution were injected per kilogram of fish weight. Once injected, fish were tagged just beneath the dorsal fin with an individually numbered floy anchor tag and released.

Most fish recovered quickly after the injection, as evidenced by their orientation to the current. Fish stayed in the vicinity of the weir for the first few hours after release before swimming upstream. Some fish initially showed a loss of equilibrium and were observed resting on the bottom. These animals were assisted until they showed normal behavior. Some fish never recovered and were found the morning after injection against the upstream screens of the collection weir. These fish were removed and discarded and were not included in the study. These fish comprised approximately 15% of the total number of fish injected.

Fish collection at the upstream weir.—The PS and PST fish were taken out of the holding ponds at the upstream weir 32–35 d after injection. Most fish arrived at the upper collection weir at least 14 d after injection, based on the appearance of tagged fish. All fish at the upstream weir were diverted into multiple holding ponds (15 × 4.6 m, 1.8 m deep) at a target density of approximately 12–16 fish/1,000 L per pond, and were held until ovulation had occurred. Ponds were loaded in succession by the date that fish arrived at the upstream weir, and as the target density was reached a new pond was used. Hence, the fish in a particular pond represented PS and PST fish that arrived over a limited period. Representative fish in each pond were checked periodically to assess their degree of ovulation. Once 60–75% of the fish were considered to have ovulated, they were diverted into a side channel and crowded to a fish lifter. Fish were lifted onto a sorting table and manually sorted such that fish with tags were identified and their tag numbers recorded.

Sample collection and thiamine and lipid analyses.—Samples were collected at both weirs; those at the lower weir were collected before ovulation, and those at the upper weir were collected after ovulation had been initiated. All fish were killed by a blow to the head prior to sampling.

At the downstream weir, tissue samples (e.g., eggs, liver, and white muscle) were taken from 12 uninjected fish exhibiting normal behavior. Samples collected at the upper weir (e.g., eggs, red and white muscle, liver, spleen, head kidney, and trunk kidney) were removed from 12 PST and 12 PS fish exhibiting normal behavior. At the upstream weir, we also sampled the same tissues from five uninjected fish that showed signs of loss of equilibrium (herein termed "wigglers") and that were clearly suffering from a thiamine deficiency (Brown et al. 2005). We compared tissue thiamine levels of wigglers with those of uninjected downstream fish and PS fish once they reached the upper weir to assess the degree of thiamine deficiency of the latter two groups.

Once collected, tissue samples were placed on dry ice and then transferred to a −80°C freezer, where they were stored until analysis for thiamine or lipid. All thiamine analyses were conducted by use of a high-performance liquid chromatography technique that separated phosphorylated (thiamine monophosphate and TPP) and unphosphorylated free thiamine forms (Brown et al. 1998). These were summed and were reported as total thiamine concentration except where noted. Lipid analysis was determined for white muscle samples by means of an in-house technique. Muscle was ground with a mechanical grinder, transferred to a Soxhlet extractor, and refluxed for 2 h in a mixture of chloroform–methanol (1:1). Lipid (%) was determined gravimetrically.

Transketolase analysis.—Tissue for transketolase analysis was collected from the liver, head kidney, and trunk kidney of the same 12 PST fish, 12 PS fish, and 6 uninjected wigglers sampled as described above. Tissues were immediately frozen on dry ice and transferred to a −80°C freezer until the time of analysis. Liver tissue was semi-thawed, cut in pieces, and homogenized in an equal volume of 0.25-M sucrose in a Potter-Elvehjem homogenizer with four up-and-down strokes at 420 rotations per minute (rpm). Head kidney and trunk kidney were homogenized with six up-and-down strokes at 420 rpm. Homogenates were diluted to give 1 g of tissue per 5 mL of suspension and then were centrifuged at 9,000 × gravity for 10 min. The supernatant fraction thus obtained was immediately used in duplicate for determination of transketolase activity as described by Smeets et al. (1971) and Tate and Nixon (1987), except for slight modifications as previously presented in Amcoff et al. (2000). The protein content was determined according to Lowry et al. (1951); bovine serum albumin was used as the standard.

Statistics.—Chi-square tests were used to test whether the number and condition (e.g., dead or alive) of fish at the upper weir were treatment related. Treatment effects on tissue thiamine levels and transketolase activity were evaluated by use of one-way analysis of variance of data that were $\log_{10}$ transformed to stabilize the variance. Comparisons among means of the transformed data were made with the Bonferroni pairwise comparison test. Lipid data were treated in the same way as thiamine, except that data were not transformed prior to testing. Correlation analyses on $\log_{10}$ transformed data were used to assess relationships between thiamine concentrations in tissues. Lineweaver–Burk plots were made based on the reciprocal of transketolase activity and TPP concentration, from which the Michaelis constant (K_m), one-half the predicted maximum transketolase activity, was derived. All tests were judged significant at the 0.05 level.

Results

Numbers of Fish Injected, Migration Upstream, and Condition at the Upstream Weir

A total of 753 fish were injected between September 11 and 14, 2001. Average fish weight for

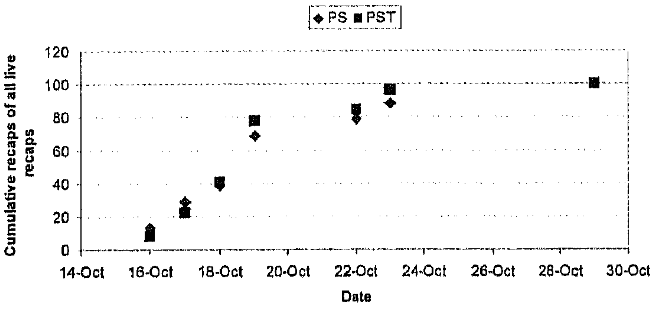

FIGURE 2.—Relationship between collection date and cumulative recaptures (dead or alive) of coho salmon injected with physiological saline (PS) or thiamine dissolved in PS (PST) and later recaptured at the upstream weir on the Platte River.

the PS and PST groups were identical at 3.72 ± 0.5 kg (mean ± SD). Of the 753 fish, 109 died in the immediate area of the lower weir and were removed. A total of 45 of these fish were from the PS treatment and 64 from the PST treatment, although no significant treatment effect was found (chi-square test: $P = 0.0671$). We assumed that these losses were of an acute nature and the direct result of the sample handling procedure. As a result, we did not adjust our expectations for fish at the upstream weir and assumed that PS and PST fish had an equal likelihood of making the upstream migration.

Individual daily counts of the number of fish injected for September 11, 12, 13, and 14 were 168, 161, 215, and 209, respectively. Similarly, the mortality levels of fish injected on these same dates were 18.5, 2.5, 15.3, and 19.6%, respectively. As a result of the mortality, a total of 644 fish remained that could potentially migrate up the river and be collected at the upstream weir.

A total of 386, or 60%, of the 644 remaining injected fish were recaptured at the upstream weir. Time of arrival and recapture of injected fish at the upstream weir was similar for both treatments (Figure 2). Of the 386 fish, 206 were from the PS treatment and 180 from the PST treatment, and no significant treatment effect was found (chi-square test: $P = 0.1857$). Of these 386 fish, 263 fish were collected live; 128 were from the PS treatment and 135 from the PST treatment, again with no significant treatment effect (chi-square test: $P = 0.6660$). The remaining 123 fish were collected dead in the bottom of the ponds at the upstream weir; 78 (63%) were from the PS treatment and 45 (37%) were from the PST treatment, and in this instance the treatment effect was highly significant (chi-square test: $P = 0.0029$).

Tissue Thiamine Levels

Location, behavior, and treatment were all associated with tissue-dependent variation in thiamine status (Table 1). The mean thiamine concentration in eggs from PS-injected fish averaged 0.767 ± 0.089 nmol/g (mean ± SE) compared to 21.925 ± 1.694 nmol/g in PST-injected fish, an increase of over 27-fold. The egg thiamine concentration of PS fish collected at the upstream weir was significantly (40%) lower than that of uninjected fish collected at the downstream weir. Coho salmon displaying wiggling behavior at the upstream weir had the lowest egg thiamine level of all groups.

The thiamine injection process also resulted in significant deposition of thiamine into white muscle. Average thiamine concentration in the white muscle of PST fish (5.894 ± 0.338 nmol/g) was over seven times that of PS fish (0.811 ± 0.126 nmol/g) (Table 1). There were no significant differences observed between thiamine levels in white muscle of uninjected fish collected at the downstream weir (0.971 ± 0.127 nmol/g) and

TABLE 1.—Summary of mean (SE) thiamine levels in eggs, liver, red and white muscle, spleen, head kidney, and trunk kidney of Platte River coho salmon collected at the lower and upper weirs. Treatments were injection with physiological saline (PS, injection with thiamine dissolved in PS (PST), or no injection (none). All fish behaved normally except uninjected fish at the upper weir (wigglers). Means followed by the same letter for the same tissue are not significantly different ($P > 0.05$); ND = not determined.

Tissue	Lower weir (none)	Upper weir PS	Upper weir PST	Upper weir None (wigglers)
Eggs	1.246 (0.091) y	0.767 (0.089) x	21.925 (1.694) z	0.458 (0.041) w
Liver	8.894 (0.363) y	6.725 (0.317) x	27.724 (1.208) z	7.086 (0.827) yx
Red muscle	ND	2.588 (0.315) y	11.443 (1.116) z	1.637 (0.155) y
White muscle	0.971 (0.127) y	0.811 (0.126) yx	5.894 (0.338) z	0.492 (0.047) x
Spleen	ND	4.589 (0.241) y	10.668 (1.073) z	4.051 (0.464) y
Head kidney	ND	4.025 (0.336) y	23.113 (1.240) z	3.764 (0.535) y
Trunk kidney	ND	3.980 (0.299) y	30.484 (2.234) z	3.654 (0.299) y

TABLE 2.—Correlation coefficients between individual tissue concentrations of total thiamine for coho salmon injected with physiological saline that were collected at the upper weir on the Platte River. Asterisks indicate significant correlations ($P < 0.05$).

Tissue	Eggs	White muscle	Red muscle	Liver	Spleen	Head kidney
White muscle	0.758*					
Red muscle	0.481	0.598*				
Liver	0.109	0.022	−0.208			
Spleen	0.260	0.373	0.231	−0.285		
Head kidney	0.442	0.600*	0.500*	−0.099	0.533	
Trunk kidney	0.389	0.620*	0.609*	−0.299	0.498	0.753*

those of PS fish collected approximately 4–5-weeks later at the upstream weir. Again, wigglers had the lowest white muscle thiamine concentration (0.492 ± 0.047 nmol/g).

Although still significant, the fourfold higher level of thiamine in the red muscle of PST fish (11.443 ± 1.116 nmol/g) than in PS fish (2.588 ± 0.315 nmol/g) was less than the sevenfold increase noted for white muscle (Table 1). The thiamine level in wigglers (1.637 ± 0.155 nmol/g) was similar to that of PS fish.

The fourfold increase in liver thiamine level resulting from the PST injection (Table 1) was intermediate to the increases observed in other tissues. The thiamine level in the livers of PST fish (27.724 ± 1.208 nmol/g) was significantly higher than that of PS fish (6.725 ± 0.317 nmol/g). There was a significant decline in liver thiamine level as fish migrated upstream, since the level in uninjected fish at the downstream weir (8.894 ± 0.363 nmol/g) was higher than that of PS fish collected at the upper weir. This decline was on the order of approximately 25%. The thiamine concentration in the livers of wigglers (7.086 ± 0.827 nmol/g) was intermediate to those of uninjected downstream fish and PS fish but was significantly lower than that of PST fish. The thiamine level in the spleens of PST fish (10.668 ± 1.073 nmol/g) was significantly higher than that of PS fish (4.589 ± 0.241 nmol/g). The spleen thiamine concentrations in wigglers (4.051 ± 0.464 nmol/g) and PS fish did not differ significantly.

Both the head kidney and trunk kidney showed a significant treatment effect (Table 1); thiamine levels for the head kidney (23.113 ± 1.240 nmol/g) and trunk kidney (30.484 ± 2.234 nmol/g) of PST fish were significantly elevated over head kidney (4.025 ± 0.336 nmol/g) and trunk kidney (3.980 ± 0.299 nmol/g) thiamine levels in PS fish. Thiamine levels in the head kidney (3.764 ± 0.535 nmol/g) and trunk kidney (3.654 ± 0.299 nmol/g) of wigglers were similar to those in PS fish.

Very few significant correlations existed between individual tissue concentrations of total thiamine; the majority of significant correlations were for PS fish at the upstream weir (Tables 2, 3). No correlations were evident among tissues from uninjected fish collected at the downstream weir.

Transketolase

Transketolase activity in the livers of upstream fish did not show any treatment- or behavior-related effects, as the level in PST fish (22.8 ± 1.5 $\text{nmol}\cdot\text{min}^{-1}\cdot\text{mg protein}^{-1}$) (mean ± SE) was not significantly different from those in PS fish (20.6 ± 1.1) or wigglers (16.9 ± 3.5). For the trunk kidney, transketolase activity in PST fish (24.1 ± 1.9) was significantly higher than activity in either PS fish (14.0 ± 2.2) or wigglers (7.8 ± 1.6); the latter two groups were not significantly different

TABLE 3.—Correlation coefficients between individual tissue concentrations of total thiamine for coho salmon injected with thiamine dissolved in physiological saline and later collected at the upper weir on the Platte River. Asterisks indicate significant correlations ($P < 0.05$).

Tissue	Eggs	White muscle	Red muscle	Liver	Spleen	Head kidney
White muscle	0.256					
Red muscle	0.416	0.201				
Liver	−0.209	0.270	0.285			
Spleen	−0.095	0.059	0.463	0.668*		
Head kidney	−0.168	0.233	0.128	0.552*	0.802*	
Trunk kidney	−0.250	−0.497	−0.058	0.377	0.592*	0.558

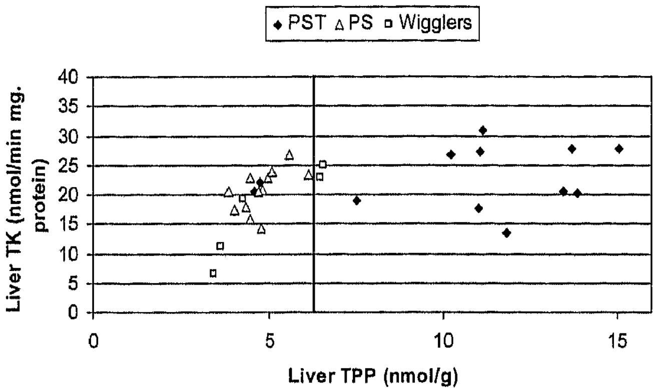

FIGURE 3.—Relationship between liver thiamine pyrophosphate (TPP) concentration and transketolase (TK) activity (nmol·min^{-1}·mg protein^{-1}) in coho salmon injected with physiological saline (PS) or thiamine dissolved in PS (PST) and uninjected coho salmon wigglers collected at the upper weir on the Platte River. The vertical line designates the Michaelis constant, K_m.

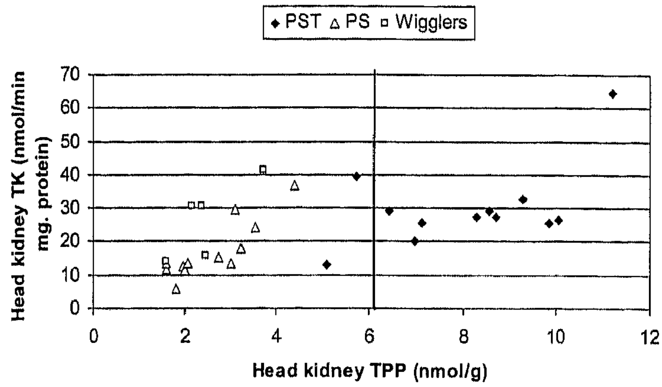

FIGURE 4.—Relationship between head kidney thiamine pyrophosphate (TPP) concentration and transketolase (TK) activity (nmol·min^{-1}·mg protein^{-1}) in coho salmon injected with physiological saline (PS) or thiamine dissolved in PS (PST) and uninjected coho salmon wigglers collected at the upper weir on the Platte River. The vertical line designates the Michaelis constant, K_m.

from each other. Transketolase activity in the head kidneys of PST fish (30.0 ± 3.6) was not significantly different from that of wigglers (26.4 ± 5.2) but was significantly elevated over that of PS fish (17.1 ± 2.5). Transketolase activity levels in the head kidneys of wigglers and PS fish were not significantly different.

Based on the transketolase activity of liver samples, tissue levels of TPP in most wigglers and PS fish were below the K_m of 6.3 nmol/g, indicating that tissue levels of TPP were less than that required for saturation of the apoenzyme (Figure 3). Similarly, for the head kidney, all of the wigglers and PS fish and two of the PST fish were below the K_m of 6.1 nmol/g for TPP (Figure 4). In contrast, relatively few fish (PS fish exclusively) had trunk kidney TPP levels that were below the K_m of 1.8 nmol/g (Figure 5).

Muscle Lipid Levels

There were no significant treatment- or behavior-related differences in muscle lipid. Levels in uninjected fish collected at the downstream weir (14.57% ± 2.01%), wigglers (12.63% ± 0.83%), PS fish (12.60% ± 0.61%), and PST fish (12.29% ± 0.87%) were all similar.

Discussion

Thiamine treatment did not affect the number of fish that migrated up the Platte River but did increase survival once fish arrived at the upstream weir. Almost twice as many fish survived from the PST treatment group than from the PS treatment group. The reason for the difference in survival is not known. The thiamine concentration in the livers of PS fish was similar to that of wigglers, a stage that thiamine-deficient fish reach just prior to exhibiting the weakness, lethargy, and equilibrium loss that precede mortality. Hence, at least some of the higher mortality we saw in PS fish was the result of low thiamine (Lehmitz and Spannhof 1977; Morito et al. 1986; Masumoto et al. 1987). The liver thiamine concentrations in PS fish (6.725 nmol/g) and wigglers (7.086 nmol/g) were apparently close to the lethal threshold, as they were lower than thiamine levels (7.11 nmol/g) measured in distressed and dying coho salmon from Platte Bay in 1999 (Brown et al. 2005). Similarly, muscle thiamine levels in uninjected fish collected at the lower weir (0.971 nmol/g) and in PS fish (0.811 nmol/g) were just slightly higher than levels measured in distressed and dying fish

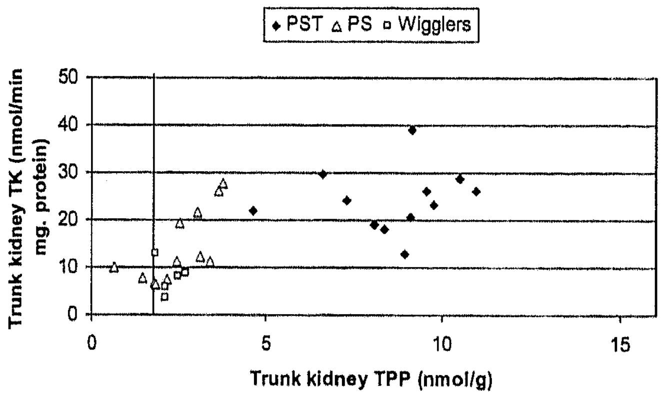

FIGURE 5.—Relationship between trunk kidney thiamine pyrophosphate (TPP) concentration and transketolase (TK) activity (nmol·min^{-1}·mg protein^{-1}) in coho salmon injected with physiological saline (PS) or thiamine dissolved in PS (PST) and uninjected coho salmon wigglers collected at the upper weir on the Platte River. The vertical line designates the Michaelis constant, K_m.

(0.76 nmol/g) from Platte Bay in 1999. We cannot rule out that the average thiamine concentrations in PS fish may have been biased high because of the death of fish with lower thiamine concentrations, although we did not sample those that died. Obviously, mortality other than that related to the thiamine deficiency also occurred, since we were only able to recover approximately 60% of the tagged fish. Some of this mortality was likely related to the sportfishing that was allowed along the 15-km migration route.

Our assessment of the impacts of a thiamine deficiency in Lake Michigan coho salmon may have been biased by thiamine-related mortality that occurred either prior to fish entry into the river or at the time of fish handling at the lower weir. Two separate mortality events exclusively involving coho salmon occurred in Platte Bay prior to fish entry into the river during the fall of 2001 (C. Pecor, Michigan Department of Natural Resources, unpublished data). Although thiamine levels were not determined for dead fish, earlier observations of dead and moribund coho salmon in Platte Bay in 1999 (Brown et al. 2005; J. Hnath, Michigan Department of Natural Resources, personal communication) ruled out infectious disease as the causal agent and implicated the low thiamine concentrations found in these fish. We saw few wigglers (<1%) among coho salmon at the downstream weir during the week when fish were injected. This would be consistent with the death of severely thiamine-deficient fish earlier in the season, before fish had entered the river. We also observed relatively high handling mortality in both thiamine- and nonthiamine-treated fish at the lower weir. Boonyaratpalin and Wanakowat (1993) also noted high handling mortality among thiamine-deficient seabass *Lates calcarifer*. The short post-injection period over which handling mortality occurred (e.g., <6 h) would have been insufficient for the injected thiamine to reverse the effects of a thiamine deficiency. Moreover, large doses of intravenous thiamine can cause vasodilation, a decrease in blood pressure, bradycardia, and respiratory arrhythmia and depression, further exacerbating the effects of the existing thiamine deficiency (Gubler 1991). As a result, the fish that entered the river and survived the injection process apparently had sufficient thiamine reserves to make the upstream migration. Nevertheless, the upstream migration still had an effect on thiamine status, and this likely affected mortality. We noted a significant decline in liver thiamine concentration, and the liver has been shown to be an important indicator of thiamine status in mammals (Gubler 1991). Moreover, approximately 20% of the fish at the upstream weir could be classified as wigglers even after many dead, and presumably severely thiamine deficient, fish had been removed from ponds by hatchery personnel.

Despite the changes in thiamine status and resulting behavior, the upstream migration had no effect on lipid levels. This contrasts with salmonines on the west coast, where 50% declines in muscle lipid have been observed during migration (M. Healey, University of British Columbia, unpublished data). Spawning salmon there, however, are apt to face more challenges than are experienced by coho salmon on the Platte River, where there are no obstructions and where the average gradient is less than 1%.

Coho salmon may be more sensitive to the effects of a thiamine deficiency than other salmonines. Liver thiamine concentration in wigglers (7.086 nmol/g) that displayed altered behavior in our study was considerably higher than that (4.8 nmol/g) associated with similar wiggling behavior in thiamine-deficient Baltic salmon (Amcoff et al. 1998). Similarly, Brown et al. (2005) reported that distressed steelhead *O. mykiss* from Lake Michigan also had a lower liver thiamine concentration (3.39 nmol/g) than coho salmon wigglers, while the liver thiamine concentration in distressed lake trout *Salvelinus namaycush* was lower still (1.75 nmol/g).

As an indication of thiamine status, tissue thiamine level seemed more sensitive than transketolase activity, although activity in some tissues appeared better able to reflect status than others. Liver thiamine level showed an obvious treatment effect that was absent for transketolase activity even though most wigglers and PS fish were below the K_m for TPP. Below the K_m, where the apoenzyme is not saturated and where first-order kinetics dominate, it would be expected that small changes in TPP should produce large changes in transketolase activity, but this was not the case. The lack of a treatment effect on liver transketolase activity contrasts with the results for trunk kidney but not head kidney. Treatment effects on trunk kidney transketolase activity mirrored those of tissue thiamine, despite the fact that most tissue TPP levels for most fish were above the K_m. However, while transketolase activity in the head kidney of PS fish was distinguishable from that of PST fish and reflected tissue thiamine level, activity was indistinguishable between wigglers and PST fish even though the thiamine levels between these two groups were clearly distinguishable. Moreover, TPP levels for both wigglers and PS fish were below the K_m, whereas the majority of PST fish had TPP levels above the K_m. Trunk kidney may be better than liver for reflecting whole-animal tissue thiamine status. Kidney transketolase activity has been used to detect a thiamine deficiency (Lehmitz and Spannhof 1977; Spannhof et al. 1978; Tiemann and Jürss 1978), whereas activity of liver tissue was unaffected by a thiamine deficiency even when clinical signs were evident (Masumoto et al. 1987). Moreover, kidney tissue homogenates were found to be about 10 times better than corresponding liver homogenates in degrading injected thiamine (Sato et al. 1993). Thus, a higher turnover and demand for thiamine in the kidney than in the liver seems likely. Although we only measured transketolase activity, other enzyme systems may also be affected and may vary in their responses. Previous studies have shown that both α-ketoglutarate dehydrogenase and transketolase activities are affected in thiamine-deficient Baltic salmon (Amcoff et al. 2000).

This study clearly demonstrated the effectiveness of injecting adults for improving total tissue thiamine status. Fourfold increases in spleen and liver concentrations, four- to sevenfold increases in muscle (red and white) concentrations, six- to eightfold increases in kidney (trunk and head) concentrations, and a 29-fold increase in egg thiamine concentration were observed approximately 1 month after injection. These tissue increases probably reflect the combined effect of (1) the extent of thiamine depletion in individual tissues prior to injection and (2) the extent to which individual tissues could accumulate thiamine when it was present in excess in the peritoneal cavity. Evidence that tissue thiamine increases in PST fish were largely the result of direct transport from the peritoneal cavity was the general lack of correlation between tissue levels. What few correlations existed were limited to hemopoietic tissue and may reflect the filtering of excess thiamine present in the bloodstream. In contrast, in PS fish, we found significant correlations between muscle and kidney tissue that were absent in PST fish, suggesting that thiamine transport was fundamentally different between PS fish and PST fish. The thiamine level in eggs of PS fish was only correlated with that of white muscle, suggesting that white muscle is a major source during the spawning run, as no such correlation was evident for uninjected fish at the downstream weir.

The highest tissue-specific increase of thiamine occurred in eggs, which indicates that eggs may be a major sink when thiamine is present in excess. Alternatively, it may indicate the extent to which thiamine was depleted in eggs as the result of thiamine deposition not keeping pace with the developing oocytes and possibly influenced by preferential allocation to other tissues. As large as the increase in egg thiamine concentration was, however, thiamine concentrations resulting from the thiamine injection were still only one-third of those measured in coho salmon from Lake Superior, where coho salmon are presumably thiamine replete (Marcquenski and Brown 1997).

Based on the thiamine concentration we used, our method of injection was relatively efficient in transferring thiamine to the egg. In another study, where Atlantic salmon were injected at a thiamine concentration that was one-seventh (7 mg/kg) of what we used, there was only a 1.5-fold increase in total egg thiamine concentration after 2–3-weeks (Ketola et al. 2000), whereas we observed a 29-fold increase. The difference likely reflects the higher dose we used, as a 20-fold increase was noted by Börjeson et al. (1999) for Atlantic salmon eggs when adults were injected at 100 mg/kg. However, the dose administered by Börjeson et al. (1999) was twice what we used, which suggests that coho salmon may respond differently from Atlantic salmon in their deposition of thiamine. Moreover, the increases that we observed after 4 weeks may not fully represent final tissue concentrations. We found far fewer significant correlations between thiamine concentrations in individual tissues for PST fish than for PS fish, suggesting that tissue thiamine concentrations in PST fish had not yet equilibrated with the injected thiamine.

Injection of broodfish, if done sufficiently before the time of ovulation, appears to be far more effective than egg immersion at increasing egg thiamine concentration and avoiding EMS. Hornung et al. (1998) used a thiamine immersion bath containing 960 mg/L but was only able to increase coho salmon egg thiamine concentrations to 2 nmol/g, an increase of approximately two-fold over controls. This level is just slightly above the approximately 1-nmol/g threshold for EMS noted by these authors. Moreover, based on preliminary investigations into the sublethal effects of a thiamine deficiency on larval lake trout, egg thiamine concentrations below 3 nmol/g are associated with increased susceptibility to predation (J. Fitzsimons, unpublished data). The average egg thiamine concentration that we achieved (22 nmol/g) was well in excess of the EMS threshold and probably exceeded thresholds for most other sublethal effects as well.

Early mortality syndrome was not evaluated in this study, but the average egg thiamine concentration measured for PS fish at the upstream weir (0.767 nmol/g) was less than the reported threshold for EMS (1 nmol/g), indicating a high potential for development of EMS (Hornung et al. 1998). Monitoring of EMS in Platte River coho salmon during 2001 appears to support this contention (Wolgamood et al. 2005).

In conclusion, while the thiamine deficiency did not appear to affect migration, there was increased adult mortality at an upstream weir; based on egg thiamine concentrations, there was a strong possibility of high larval mortality as well.

Acknowledgments

The authors would like to acknowledge the generous financial support of the Fish and Wildlife Restoration Act. We would also like to thank S. Blunt of the Department of Fisheries and Oceans (DFO) for lipid analysis and C. Boyko of DFO for his assistance in the field. The financial support received from the Tryggers Foundation and the Swedish Foundation for International Cooperation and Higher Education is also gratefully acknowledged. Reference to trade names does not imply endorsement by the U.S. Government.

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