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Thiamine (Vitamin B₁) Concentrations in Salmon (*Salmo salar*), Brown Trout (*Salmo trutta*) and Cod (*Gadus morhua*) from the Baltic Sea

During recent decades several Baltic fish species have been affected by poor reproductive success. The sea-run Baltic salmon populations are affected by the M74 syndrome, an early life-stage mortality associated with low thiamine (vitamin B₁) concentrations in the offspring. In order to study whether sea-run brown trout with symptoms and mortalities similar to M74 also suffer from low thiamine concentrations, analyses of thiamine were performed. Also, tissues of Baltic cod were analyzed for their content of thiamine. This study confirms that Baltic salmon that are affected by M74 suffer from low thiamine levels and that the temporal onset of the development of M74 may be correlated to the thiamine concentration in the eyed egg. Also, thiamine concentrations of muscle and ovaries from females that produced offspring with M74 were significantly lower than those of females that produced healthy progeny, which indicates that M74 may be a maternally transmitted thiamine deficiency. Preliminary data from sea-run Baltic brown trout showed that yolk-sac fry with symptoms that resembled those of M74—lethargy, darkening of skin and 100% mortality—had low mean thiamine concentrations (0.14 nmol g⁻¹ in 1994 and 0.34 in 1998) compared with those of healthy progeny (4.4 nmol g⁻¹ in 1994 and 3.9 in 1998). This indicates that some family groups of Baltic brown trout may be affected by a reproduction disorder related to thiamine deficiency. In spawning Baltic cod, hepatic and ovarian thiamine concentrations showed great variance, with values ranging from 0.56 to 4.7, and 3.8 to 30 nmol g⁻¹, respectively. Whether Baltic cod also suffer from mortality associated with a thiamine deficiency is not known.

INTRODUCTION

During recent decades several fish species inhabiting the Baltic Sea have been afflicted by reproductive disturbances (1). The sea-run Atlantic salmon (*Salmo salar*) of the Baltic Sea has been affected by a lethal early-life stage disorder called M74 syndrome (2–4). Low concentrations of thiamine (vitamin B₁) in offspring as well as adult fish have been suggested to play a major role in the development of M74, and Baltic salmon on their spawning migration have also displayed M74-associated disturbances, manifested mainly by wiggling and a lack of coordination, which in severe cases may result in death (5). Female broodfish demonstrating wiggling behavior have been shown to have low ovarian thiamine content and to produce high numbers (98%) of offspring affected by M74 (5). Therapeutic treatment of brood fish by injection of thiamine has been shown to counteract the development of M74 (6). Offspring of salmon that develop M74 also respond positively to thiamine treatment and a complete loss of the symptoms and mortalities associated with M74 may be achieved by immersion of the affected progeny in thiamine-enriched water, providing further evidence of the involvement of thiamine in the pathogenesis (7, 8).

Poor reproductive success also affects wild populations of anadromous Baltic brown trout (*Salmo trutta*). Vallin (9) dem-

onstrated variability in survival of progeny from different family groups of sea-run brown trout in natural habitats on the Swedish island of Gotland. Reduced embryo survival has also been reported for brown trout from Estonian rivers (10) and from the Polish river Vistula (11). The sporadic occurrence in Swedish compensatory rearing stations of family groups of brown trout that exhibit symptomatology and mortalities similar to M74 in salmon, and their positive response to thiamine treatment (12), indicates that brown trout may also be affected by an M74-like reproduction disorder linked to a thiamine deficiency.

The spawning stock biomass of Baltic cod (*Gadus morhua*) has shown a downward trend since 1985 (13). Besides heavy exploitation by commercial fishing, this decline may involve low salinity, increased temperature, oxygen depletion (13, 14), female size (15), predation (16) or the contribution of pollutants that generate free radicals (17). In addition, a prolonged spawning period has been reported; the normal March to June period now extends from March to October (18). Baltic cod eggs and yolk-sac fry were bathed in a solution of thiamine, but without improving survival (19, 20).

Thiamine is a water-soluble essential compound mainly produced by microbes and microalgae (21). Thiamine is phosphorylated to thiamine mono-, di- and tri-phosphate, and the di-phosphate (TDP) is a cofactor for three TDP-dependent enzymes, necessary in carbohydrate metabolism. Thiamine deficiencies causing high mortalities in farmed fish have been reported since the early 1940s (22), but when considering fish populations in the wild the diagnosis of thiamine deficiency is considerably more difficult. The Cayuga Syndrome (CS), which affects larval Atlantic salmon (*Salmo salar*) in the New York Finger Lakes, and another disorder which affects the progeny of several salmonids of the Great Lakes Basin, the Early Mortality Syndrome (EMS), have both been suggested to be naturally occurring thiamine deficiencies caused by consuming alewife (*Alosa pseudoharengus*) or rainbow smelt (*Osmerus mordax*), both of which contain high proportions of thiaminase I (23–27). Thiaminase I (EC 2.5.1.2) is an enzyme that actively destroys thiamine by a base-exchange reaction (28). Whether the M74 syndrome is a naturally occurring thiamine deficiency, and whether other fish species from the Baltic Sea, like brown trout and cod, are affected by feeding on prey that contain thiaminase I, still remains to be elucidated.

The aim of this study was to analyze and compare thiamine concentrations in different tissues and in offspring of sea-run Baltic salmon and brown trout and to compare the results with yolk-sac fry survival. Tissues of Baltic cod were also analyzed for their thiamine content.

MATERIAL AND METHODS

Fish Samples

The fish samples were collected from different geographical locations in Sweden and the Baltic Sea between 1994 and 1998. For the location of the sampling sites, see Bengtsson et al. (29). All adult fish were weighed and killed by a blow to the head

Table 1. Mean thiamine concentrations (nmol g⁻¹ ± SD) in tissues of female (F) and male (M) adult sea-run and farmed Baltic salmon (*Salmo salar*). "Normal" and "wiggling" indicate normally behaving and wiggling sea-run brood fish, respectively. "M74" and "healthy" indicate whether offspring from sea-run brood fish developed M74 or were healthy, and "farmed" indicates healthy farmed offspring. Significant differences between groups are indicated by different letters after the means.

Location and sampling	n	Status	Sex	Weight	Hepatic thiamine (nmol g ⁻¹)	Ovarian thiamine (nmol g ⁻¹)	White muscle thiamine (nmol g ⁻¹)
Luleälven, Oct 6, 1994	8	Normal	F	9.9 ± 3.6	6.2 ± 1.5z	1.0 ± 2.5 ¹ z	
Luleälven, Oct 6, 1994	12	Wiggling	F	9.1 ± 3.2	4.7 ± 1.0y(*)	0.27 ± 0.059y(*) ¹	
Luleälven, Oct 6, 1994	2	Normal	M	6.7 ± 1.1	12 ± 2.4		
Luleälven, Oct 6, 1994	2	Wiggling	M	4.5 ± 0.92	4.7 ± 0.44		
Dalälven, Nov 6, 1996	6	M74	F	4.8 ± 1.4	5.3 ± 0.74	0.59 ± 0.1y(**) ²	1.4 ± 0.24y(**)
Dalälven, Nov 6, 1996	6	Healthy	F	6.4 ± 2.1	6.2 ± 0.95	1.7 ± 0.56z ²	2.4 ± 0.59 z
Dalälven, Nov 8, 1995	10	Farmed	F	2.8 ± 0.50	9.5 ± 2.8		
Dalälven, Nov 8, 1995	4	Farmed	M	2.7 ± 0.62	19 ± 7.7		

¹ Ovaries dissected approximately three weeks before stripping.

² Unfertilized eggs.

Table 2. Mean thiamine concentrations (nmol g⁻¹ ± SD) in newly fertilized eggs, eyed eggs and yolk-sac fry of sea-run and farmed Baltic salmon (*Salmo salar*) from different Swedish rivers. The yolk-sac fry were sampled at 1–10, 30–40, or 150–180 posthatch degree-days (d°C). "M74" and "healthy" indicate whether offspring from family groups of sea-run brood fish developed M74 or were healthy, and "farmed" indicates healthy farmed offspring. Number of analyzed individuals (n) in parentheses after the thiamine concentration.

River, hatching year	n	Status	Eggs (nmol g ⁻¹)		Yolk-sac fry (nmol g ⁻¹)		
			Fertilized eggs	Eyed eggs	1–10 d°C	30–40 d°C	150–180 d°C
Dalälven, 1994	18	M74		0.36 ± 0.24			
Dalälven, 1995	7	M74		0.19 ± 0.13		0.12 ± 0.09	0.12 ± 0.12 (3)
Dalälven, 1996	4	M74		0.21 ± 0.27			
Dalälven, 1996	15	M74		0.62 ± 0.13			
Dalälven, 1997	4	M74					0.74 ± 0.12
Dalälven, 1998	11	M74		0.59 ± 0.18			
Luleälven, 1995	9	M74		0.27 ± 0.10	0.20 ± 0.12 (4)	0.18 ± 0.09 (4)	
Luleälven, 1996	15	M74	1.1 ± 0.25			0.49 ± 0.20	0.26 ± 0.12 (6)
Dalälven, 1995	8	Healthy		2.4 ± 2.7		1.7 ± 1.7	
Dalälven, 1996	13	Healthy		1.6 ± 1.0			
Dalälven, 1998	18	Healthy		2.5 ± 1.7			
Luleälven, 1995	4	Healthy	2.3 ± 0.86				
Luleälven, 1995	8	Healthy		2.0 ± 0.8			
Luleälven, 1995	16	Healthy		1.1 ± 0.89			
Luleälven, 1996	4	Healthy			1.1 ± 0.80	1.0 ± 0.50	
Luleälven, 1997	4	Healthy			1.7 ± 0.74	1.4 ± 0.44	
Klarälven, 1996	4	Healthy	7.4 ± 0.50				
Lagan, 1996	8	Healthy		2.9 ± 0.41			
Dalälven, 1995	5	Farmed		17 ± 4.1		5.0 ± 0.71	

and dissected tissues were rapidly put in liquid nitrogen or between slabs of dry ice and then transferred to a -70°C freezer where they were stored for a maximum of 6 months before thiamine analysis. The Baltic salmon progeny were anaesthetized in MS-222 (Sandoz Ltd., Basel, Switzerland; 2 min in 140 mg L⁻¹) and then immediately frozen in liquid nitrogen or between slabs of dry ice. When MS-222 was not available, eggs and yolk-sac fry were put in zip-lock® bags and killed by rapid freezing in liquid nitrogen or between slabs of dry ice, and stored as described above (any exceptions being described in the text). Embryos were sampled as newly fertilized eggs, eyed eggs (the developmental stage when the eye pigment becomes visible through the chorion) or as yolk-sac fry. Mortality rates for Baltic salmon progeny are described as M74 or healthy, i.e., absence of M74-related mortality. For Baltic brown trout mortality rates for the yolk-sac fry stage are presented when available, and groups that demonstrate symptoms that resemble those of M74 are classified as M74. Sea-run salmon and brown trout are fish reared to the smolt stage in a hatchery and released to their original river for feeding migration to the Baltic Sea. As controls we used farmed salmon, which were reared in a net-pen and fed a commercial feed. Due to difficulties in raising cod embryos, no mortalities are presented for the cod family groups (30). For age determination cumulative posthatch degree-days were used (d°C). Samples were transported to the laboratory on dry ice for

thiamine analysis or for storage in a -70°C freezer. In order to evaluate whether the thiamine concentrations of eyed eggs of Baltic salmon were correlated to the onset of M74-development, the thiamine concentrations of eyed eggs were plotted against the posthatch degree-days when the first M74-symptoms were manifested in each individual family group.

Baltic salmon

In Luleälven, October 1994, livers and ovaries from sea-run females (n = 20) were sampled approximately 3 weeks before ripening (Table 1). Of the sampled females, 12 displayed wiggling and uncoordinated behavior (5, 31), and 8 behaved normally. In addition, livers were sampled from wiggling and normally-behaving sea-run males on the same occasion. From Dalälven, sea-run broodfish were sampled in November 1996, in connection with stripping the females for eggs. The females were weighed, killed and sampled for liver, white muscle and unfertilized eggs. Based on prognostic hatching; i.e. hatching of a small batch of eggs (n = 200) from each female to screen in advance for family groups developing M74, females that produced offspring with M74, and females that produced viable offspring were selected for thiamine analysis. In November 1995, samples were taken from adult farmed females and males of Dalälven origin, for determination of hepatic thiamine concentrations. In addition, farmed females originating from Dalälven were sampled for

liver, heart, red and white muscle, whole brain, head and main kidney (anterior and posterior part), ripened eggs, and intestine posterior of the pyloric caecae, for determination of thiamine concentrations. The intestine was rinsed in 0.9% sodium chloride in order to remove digested food.

Eyed eggs from sea-run females originating from Dalälven were sampled in 1994, 1995, 1996, and 1998 (Table 2). In addition, yolk-sac fry from sea-run females from Dalälven were sampled in 1995 and 1997 and eggs and yolk-sac fry from farmed females from Dalälven were sampled in 1995.

Newly fertilized eggs, eyed eggs, and yolk-sac fry from Luleälven were sampled during the hatching years 1995 and 1996 (Table 2). In 1997, yolk-sac fry from Luleälven were sampled. In addition, newly fertilized eggs from Klarälven were sampled in autumn 1995, and eyed eggs were collected in February 1996 from the river Lagan, on the Swedish west coast.

Baltic brown trout

Sea-run adult brown trout originating from river Mörrum were stripped of eggs and killed in October, 1995, and samples of liver and newly fertilized eggs were taken (Table 3). From river Gullspång, which empties into Lake Vänern, newly fertilized eggs of sea-run origin were sampled in November 1995. From river Lagan, on the Swedish west coast, family groups of eyed eggs were sampled in February 1996. In October 1996, family groups of newly fertilized eggs were collected from Emån and put in zip-lock® plastic bags which were rapidly frozen between slabs of ice in a -25°C freezer after which they were stored in a -70°C freezer until analysis. Yolk-sac fry originating from 3 different streams on Gotland were collected in May 1997. From Dalälven in May 1994 and 1998, family groups of yolk-sac fry were sampled at ≈100 d°C. Of these, two groups in both 1994

and 1998 demonstrated symptoms that resembled those of M74, such as uncoordinated behavior, lethargy and darkening of skin, and all individuals died before 250 d°C.

Baltic cod

From Baltic Sea areas S and SE of Gotland (ICES statistical subdivision 28; for figure see Karlsson et al., 32), spawning females and males were caught by trawling in June 1996 (Table 4). The cod were either transported alive to the laboratory at Ar, Gotland, where they were killed and dissected for liver and ovaries, or were killed and dissected onboard the fishing vessel. Tissues were immediately frozen between slabs of dry ice. In June 1997, spawning females were caught in the same area and dissected for liver and ovaries onboard the fishing vessel. Also in June 1997, spawning females and males were caught in the southern Baltic proper (ICES statistical subdivisions 25 and 26) by trawling, and sampled for liver and ovaries for analysis of thiamine concentrations.

Thiamine analysis

Thiamine was extracted using acid and enzymatic hydrolysis based on the method of Roser et al., (33). Each sample (3–15 g) was homogenized on an Ultra-turrax mixer (TP18-10, Janke & Kunkel KG, Germany) and extracted using 0.1 M HCl at 121°C for 30 min. The extract was cooled to 23°C and the pH adjusted to 4.0 by adding 2.0 M sodium acetate buffer (pH 6.1). A suspension of Taka-Diastase in water was added to the extract at a ratio of 0.1 g Taka-Diastase g⁻¹ sample. After incubation at 45°C for 4 hrs, the extract was cooled to 23°C and filtered through Munktell V120H folded paper (Munktell, Grycksbo, Sweden). The conversion efficiency of the Taka-Diastase preparation was checked by analysis of known amounts of thiamine and thiamine diphosphate (TDP) added to different types of tissue samples (liver, egg and yolk-sac fry). The recovery of added thiamine and TDP was between 96–99%. The coefficient of variation ($V = 0.012$) of the method was determined by consecutive analysis of 2 different tissue samples. All samples were analyzed in duplicate and presented on a wet weight basis (ww).

The thiamine in the extract was converted to the fluorescent thiochrome compound using an automated pre-column derivation technique. An ASPEC liquid-handling robot with a Model 401 dilutor (Gilson, Villiers-le-Bel, France) was used to mix 2.0 ml of the sample extract with 1.1 ml of derivation reagent (3 mM K₃Fe(CN)₆ in 4 M NaOH). A stream of air was used to ensure mixing and the derived extract was subjected to a HPLC after 80 sec. The HPLC system consisted of an LC-10AD pump, an RF-

551 spectro-fluorometric detector, a Chromatopac C-R5A integrator (all from Shimadzu, Tokyo, Japan) and a Model 7010 injector (Rheodyne, Cotati, CA, US) equipped with a 20-μL loop. The column (5 μm packing material; 150 mm x 4.1 mm i.d.) was a polymer-based PRP-1 (Hamilton Co., Reno, NV, US). Mobile phase was a 35% (v/v) mixture of methanol in water adjusted to pH 9.0–9.1 with a 25% solution of ammonia and degassed on an ultrasonic bath. The flow was 0.8 ml min⁻¹. All analytical work was performed in laboratories protected against ultraviolet radiation. Detection was performed at 366 nm excitation wavelength and 435 nm emission wavelength.

Thiamine concentrations were calculated using external stand-

Table 3. Mean thiamine concentrations (nmol g⁻¹ ± SD) in liver, eyed eggs (E.E.), newly fertilized eggs (F.E.) and yolk-sac fry (YSF) of sea-run Baltic brown trout (*Salmo trutta*) caught at different locations in the Baltic from 1994–98. Yolk-sac fry (YSF) were sampled at 100 or 250 posthatch degree-days (d°C). "Normal" indicates that normally behaving sea-run brood fish produced healthy offspring. "Healthy" and "M74" indicate offspring that developed into healthy yolk-sac fry or into family groups with symptoms and mortalities similar to those observed in Baltic salmon (*Salmo salar*) with M74. Mortalities from eyed-egg stage to swim-up fry; d.n.a. = data not available.

River, sampling occasion	n/Status	Sample	Mortality	Thiamine nmol g ⁻¹
R. Mörrum, Oct 30, 1995	15/Normal	Liver	–	6.4 ± 1.2
R. Mörrum, Oct 30, 1995	15/Normal	F.E.	8%	5.8 ± 1.1
R. Gullspång, Nov 10, 1995	8/Healthy	F.E.	1%	4.7 ± 0.62
R. Lagan, Feb 5, 1996	5/Healthy	E.E.	2.2%	5.0 ± 0.56
Emån, Oct 23, 1996	3/Healthy	F.E.	d.n.a.	5.0 ± 2.0
Gotland, May, 1997 ¹	19/Healthy	YSF, 250 d°C	1.2% ²	5.5 ± 1.4
Dalälven, May, 1994	4/Healthy	YSF, 100 d°C	3%	4.4 ± 3.2
Dalälven, May, 1998	5/Healthy	YSF, 100 d°C	3%	3.9 ± 2.1
Dalälven, May, 1994	2/"M74" ³	YSF, 100 d°C	100%	0.14 ± 0.065
Dalälven, May, 1998	2/"M74"	YSF, 100 d°C	100%	0.34 ± 0.32

¹ Caught in three small streams, Arån, Bångån and Hultungån, on the island of Gotland.

² Mortalities from hatching to swim-up stage.

³ Family groups manifesting lethargy, darkening of skin and 100% mortality. The first symptoms were observed at approximately 100 d°C and all affected individuals were dead at ≈ 250 d°C.

Table 4. Mean thiamine concentrations (nmol g⁻¹ ± SD) in tissues from spawning female (F) and male (M) Baltic cod (*Gadus morhua*) caught in 1996–1997 around the island of Gotland (ICES statistical rectangle 28; for map see Karlsson et al. this volume; 32) and from the Southern Baltic proper (ICES statistical rectangles 25 and 26).

Location and sampling	n	Sex	Liver nmol g ⁻¹	Gonads nmol g ⁻¹
ICES 28 June 1996	10	F	2.0 ± 1.4	12 ± 7.4
ICES 28 June 1997	9	F	1.4 ± 0.77	19 ± 9.7
ICES 25, 26 June 1997	12	F	0.98 ± 0.21	12 ± 6.1
ICES 28 June 1996	2	M	4.3 ± 2.6	4.5 ± 1.8
ICES 25, 26 June 1997	3	M	3.8 ± 0.68	7.6 ± 1.0

ards of thiamine that were subjected to the extraction and derivation steps mentioned above. The thiamine standard was prepared daily in 0.01 M HCl and used immediately. Integrated peak areas were corrected for thiamine originating in the enzyme preparations by subtracting the peak areas of the blanks from the samples. Reference material was analyzed in parallel with the samples to ensure reliable results. The thiamine hydrochloride (96.6% dw) used in the thiamine analysis was supplied by Fluka A.G. (Buchs SG, Switzerland). Taka-Diastase was sup-

plied by Pfaltz & Bauer (Chemicon, Stockholm, Sweden). All other chemicals were of analytical grade and the water was of millipore quality (Milli-QPLUS 185, Millipore S.A., Molsheim, France).

Statistics

When testing for differences in thiamine concentrations between viable and M74 groups Student's *t*-test was applied. The normality was checked by using Z-score histograms. To test whether the thiamine concentrations in Baltic salmon family groups that developed M74 correlated with the first signs of M74-development, simple regression analysis was used and p-values were calculated using analysis of variance. The thiamine concentrations at the eyed-egg stage were subjected to Log_{10} transformation ($Y' = \text{Log}_{10}(\text{Thiamine Concentration} + 1)$) and plotted against the posthatch degree-days ($d^{\circ}\text{C}$) when the first M74-symptoms were observed. In all testing, the work of Zar (34) was consulted, and the statistics were calculated using the StatView 4.5 data analysis system (Abacus Concepts, Inc., Berkeley, California). The significance level was set at 0.95 and presented as a p-value with the attached symbol; $p \leq 0.05$ (*), $p \leq 0.01$ (**) and $p \leq 0.001$ (***)

RESULTS

Thiamine Concentrations

Adult Baltic salmon

Hepatic and ovarian thiamine concentrations of wiggling females from Luleälven were significantly ($p \leq 0.05$) lower than those of normally-behaving females (Table 1). The wiggling and normally behaving males had mean hepatic thiamine concentrations of 4.7 and 12 nmol g^{-1} , respectively. Of the adult brood fish from Dalälven in 1996, females producing M74-affected progeny had significantly ($p \leq 0.01$) lower ovarian and white muscle thiamine concentrations than females with viable offspring. Hepatic thiamine concentrations were 6.2 and 5.3 nmol g^{-1} in normal and wiggling individuals, respectively.

Farmed females and males from Dalälven sampled in 1995

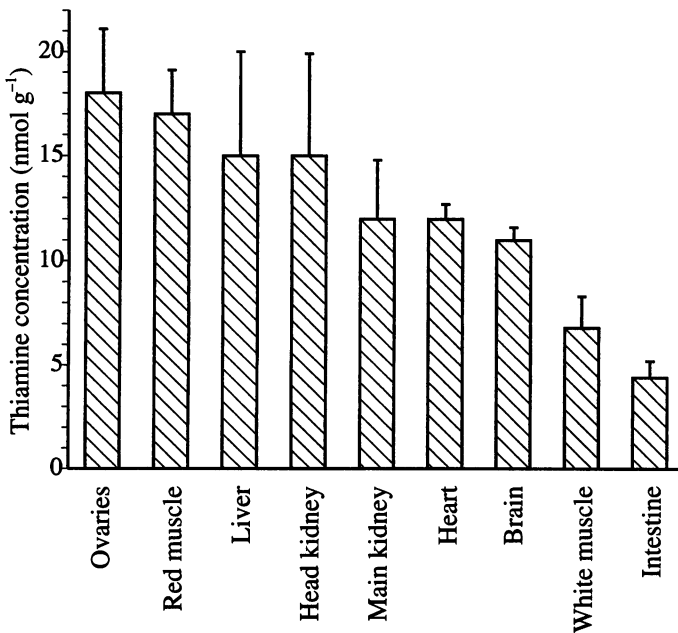
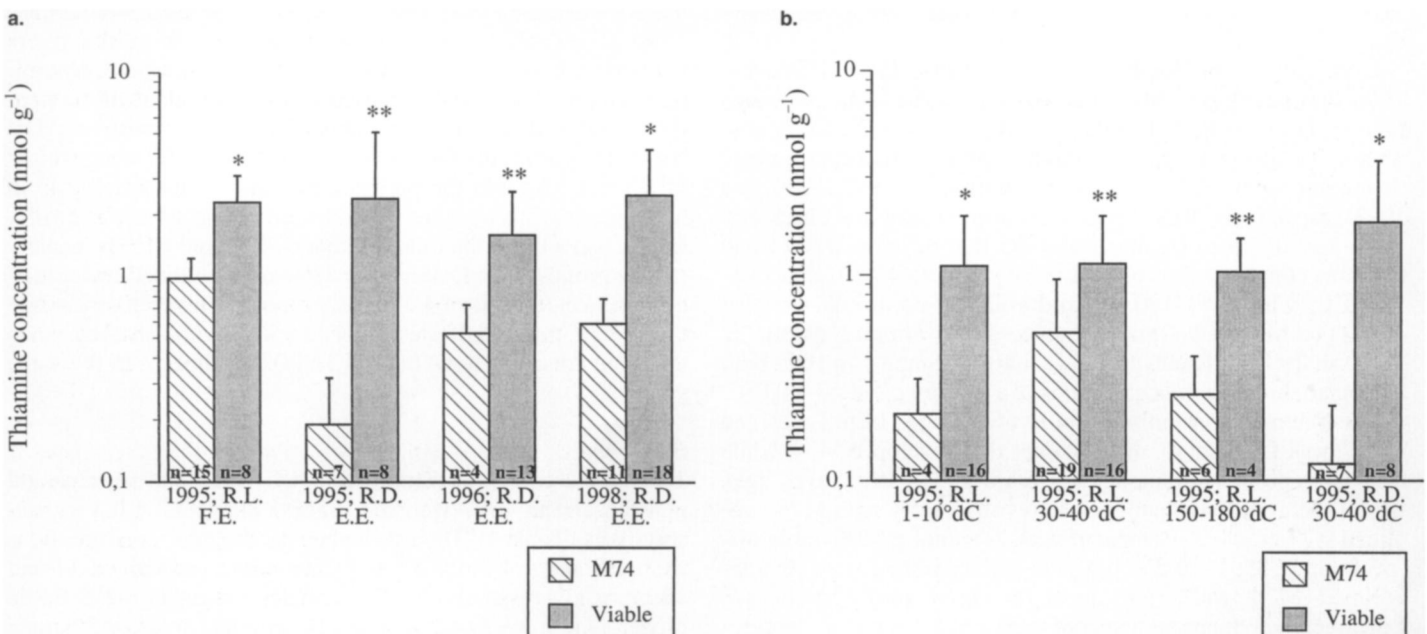
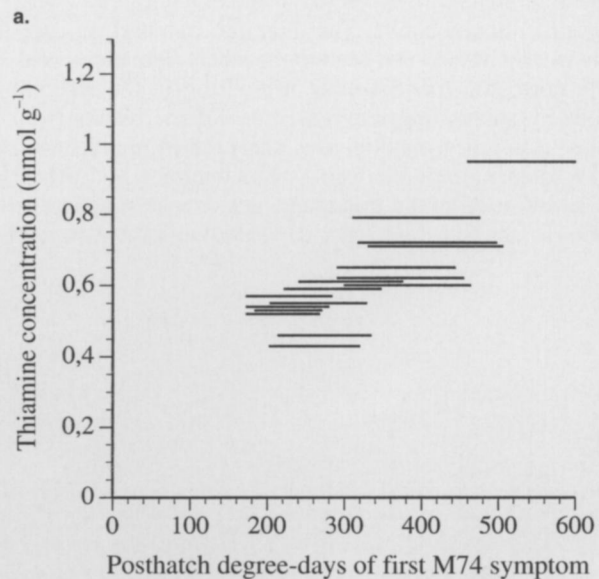


Figure 1. Mean thiamine concentrations in $\text{nmol g}^{-1} \pm \text{SD}$ of tissues dissected from ripened farmed Baltic salmon (*Salmo salar*) females ($n = 4$) originating from Dalälven in 1996. Samples were taken of ovaries, red muscle, liver, head and main kidney, heart, brain, white muscle and intestine after the pyloric caeca.

Figures 2a–b. Mean thiamine concentrations in $\text{nmol g}^{-1} \pm \text{SD}$ in offspring of Baltic salmon (*Salmo salar*) from different Swedish rivers in 1995–1998. Figure 2a; newly fertilized eggs (F.E.) and eyed eggs (E.E.) sampled in 1995, 1996, and 1998 from the rivers Luleälven (R.L.) and Dalälven (R.D.). Figure 2b; yolk-sac fry sampled in 1995 at 1–10, 30–40, and 150–180 posthatch degree-days ($d^{\circ}\text{C}$) from the rivers Luleälven (R.L.) and Dalälven (R.D.).



Figures 3a–b. Thiamine concentrations in nmol g^{-1} in eyed eggs of Baltic salmon (*Salmo salar*) versus posthatch degree-days of first observed M74-symptoms (indicated by the beginning of the line) and the duration of M74 in each family group (indicated by the end of the line).
 Figure 3a; Eyed eggs ($n = 16$) from Dalälven that demonstrated 100% mortality in the hatching year 1996.
 Figure 3b; Eyed eggs from Dalälven in the hatching year 1998. Of the family groups ($n = 11$) 8 displayed total mortality while the three family groups with the highest thiamine concentrations (0.71, 0.73 and 0.98 nmol g^{-1}) showed partial development of M74 with 25–50% mortalities.



showed mean hepatic thiamine concentrations of 9.5 and 19 nmol g^{-1} , respectively. This corresponded to those in farmed females from Dalälven sampled in 1996 that had a mean weight of $2.2 \pm 0.45 \text{ kg}$ and contained $15 \pm 5.0 \text{ nmol g}^{-1}$ liver thiamine (Fig. 1). The tissues demonstrating the highest mean thiamine concentrations were dissected; ripened ovaries ($18 \pm 3.1 \text{ nmol g}^{-1}$), red muscle ($17 \pm 2.1 \text{ nmol g}^{-1}$), liver, head kidney ($15 \pm 4.9 \text{ nmol g}^{-1}$) while the heart and main kidneys contained 12 ± 0.70 and $12 \pm 2.8 \text{ nmol g}^{-1}$, respectively. The brain showed a mean thiamine concentration of $11 \pm 0.62 \text{ nmol g}^{-1}$ and the lowest variation of the sampled tissues. The white muscle and the intestine, sampled behind the pyloric caecae, were the tissues with the lowest thiamine content, 6.8 ± 1.5 and $4.4 \pm 0.80 \text{ nmol g}^{-1}$, respectively.

Eggs and yolk-sac fry of Baltic salmon

In general, the thiamine content of family groups of salmon eggs and yolk-sac fry that developed M74 were significantly lower than in healthy offspring. When comparisons between groups were possible (i.e. when progeny from the same river, year class, and developmental stage were available) the groups developing M74 contained less than 50% of that of viable groups (Figs 2a–b).

Eyed eggs from Dalälven for years 1994, 1995, 1996, and 1998 that developed M74 had mean thiamine concentrations ranging between 0.19 and 0.62 nmol g^{-1} (Table 2). Compared with those of eyed eggs developing normally that had a mean thiamine content of 2.4 , 1.6 and 2.5 nmol g^{-1} in 1995, 1996, and 1998, respectively, these levels were approximately 4 times less. Yolk-sac fry from Dalälven in 1995 that developed M74 had thiamine concentrations of 0.12 nmol g^{-1} at $30\text{--}40 \text{ d}^\circ\text{C}$ and $150\text{--}180 \text{ d}^\circ\text{C}$, while $150\text{--}180 \text{ d}^\circ\text{C}$ old yolk-sac fry from 1997 that developed M74 had a thiamine content of 0.74 nmol g^{-1} . Viable yolk-sac fry from Dalälven and Luleälven sampled in 1995 both had mean thiamine concentrations of 1.7 nmol g^{-1} at $30\text{--}40 \text{ d}^\circ\text{C}$.

The thiamine concentration in fertilized eggs from Luleälven (1995) was 1.1 nmol g^{-1} in the groups that developed M74, while the viable progeny contained 2.3 nmol g^{-1} . In 1995, eyed eggs from Luleälven that developed into yolk-sac fry with M74 contained 0.27 nmol g^{-1} , compared with 2.0 nmol g^{-1} for viable offspring. In 1995, $1\text{--}10 \text{ d}^\circ\text{C}$ old yolk-sac fry from groups that developed M74 and from normally developing groups for Luleälven had thiamine levels of 0.20 and 1.1 nmol g^{-1} , respec-

tively. In the corresponding groups at $30\text{--}40 \text{ d}^\circ\text{C}$ the thiamine contents were 0.18 and 1.1 nmol g^{-1} , respectively. In family groups from Luleälven (1995), that developed M74, mean thiamine concentrations were 0.49 at $30\text{--}40 \text{ d}^\circ\text{C}$ and 0.26 nmol g^{-1} at $150\text{--}180 \text{ d}^\circ\text{C}$. Viable yolk-sac fry from Luleälven in 1996 showed a decline in thiamine concentration from 1.7 at $30\text{--}40 \text{ d}^\circ\text{C}$ to 1.0 at between 150 and $180 \text{ d}^\circ\text{C}$, and in 1997 old yolk-sac fry groups at between 150 and $180 \text{ d}^\circ\text{C}$ demonstrated a mean thiamine level of 1.4 nmol g^{-1} .

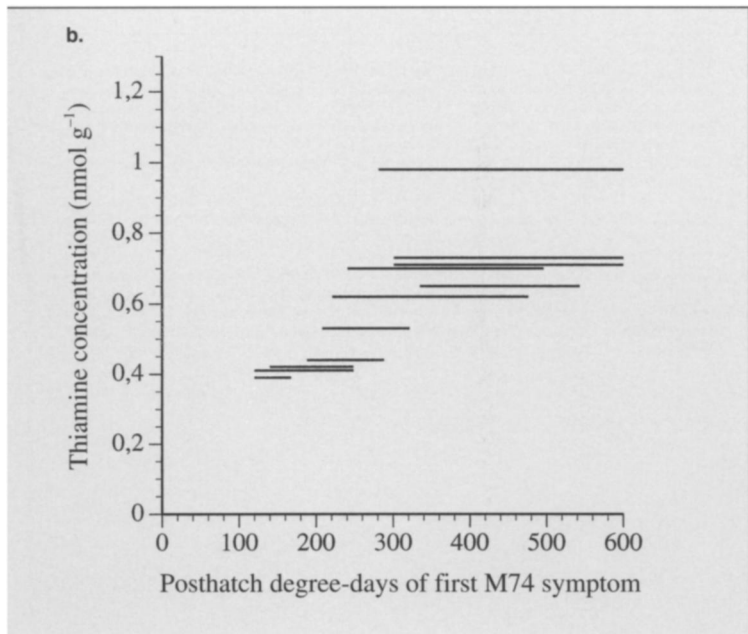
The eyed eggs from river Lagan, on the Swedish west coast, contained 2.9 nmol g^{-1} and the fertilized eggs from Klarälven showed a considerably higher mean thiamine concentration of 7.4 nmol g^{-1} . The offspring of farmed Dalälven origin showed the highest thiamine concentrations, 17 nmol g^{-1} at the eyed egg stage, which declined to 5.0 nmol g^{-1} at the yolk-sac fry stage ($150\text{--}180 \text{ d}^\circ\text{C}$).

Baltic brown trout

The brown trout from river Mörrum had a mean weight of $6.5 \pm 0.92 \text{ kg}$ and no unusual mortality was recorded in any investigated family group. The hepatic thiamine concentration was 6.4 nmol g^{-1} and the newly fertilized eggs contained $5.8 \text{ nmol thiamine g}^{-1}$ (Table 3). Newly fertilized eggs from the rivers Gullspång, Lagan and Emån showed mean thiamine concentrations ranging between 4.7 and 5.0 nmol g^{-1} and none of the family groups suffered from any abnormal embryo mortality. The brown trout from the three rivers on Gotland had a mean weight of $2.8 \pm 1.5 \text{ kg}$ and the prehatch mortality and mortality from hatching to swim-up were 4.5 and 1.2% , respectively. The yolk-sac fry had a mean thiamine content of 5.5 nmol g^{-1} . The healthy family groups from Dalälven in 1994 and 1998 had mean thiamine concentrations of 4.4 and 3.9 nmol g^{-1} , respectively, while the groups that manifested M74-like symptoms showed mean thiamine concentrations of 0.14 and 0.34 nmol g^{-1} in the same years.

Baltic cod

The female cod from waters around Gotland that were caught in 1996 and in 1997 weighed 2.5 ± 1.2 and $2.2 \pm 1.1 \text{ kg}$, respectively (Table 4). Their mean hepatic thiamine concentrations were 2.0 and 1.4 nmol g^{-1} and their ovaries contained 12 and 19 nmol g^{-1} , respectively. The females caught in the S Baltic proper ($4.4 \pm 3.9 \text{ kg}$) had mean hepatic and ovarian thiamine



concentrations of 0.98 and 12 nmol g⁻¹. In males from Gotland (1.0 ± 0.3 kg), the mean thiamine concentration was 4.3 nmol g⁻¹ in the liver and 4.5 nmol g⁻¹ in the testes. The males from S Baltic proper (0.8 ± 0.5 kg) displayed hepatic and testes thiamine concentrations of 3.8 and 7.6 nmol g⁻¹, respectively.

Relationship between initial thiamine concentration in eyed eggs of Baltic salmon and development of M74

A significant ($p \leq 0.05$) dose-dependent relationship was observed between the thiamine concentration of eyed eggs of Baltic salmon and the first onset of M74 symptoms at the yolk-sac fry stage in samples from Dalälven for the hatching years 1996 and 1998. Thus, a significant ($p \leq 0.01$ – 0.001) thiamine dose-dependent onset of M74 could be demonstrated (regression equation for 1996, $y = 0.11 + 3.76 \times 10^{-4}x$; $R^2 = 0.779$; and for 1998, $y = 0.084 + 5.24 \times 10^{-4}x$; $R^2 = 0.685$). The onset and duration of M74 for each of the above-described family groups versus individual thiamine concentrations are presented in Figures 3a–b, where 3 family groups from Dalälven in 1998 (Fig. 3b) suffered from partial M74 development with mortalities between 25 and 50%. These three family groups also had the highest thiamine concentrations (0.71; 0.73; and 0.98 nmol g⁻¹) of the family groups that developed M74 in 1998.

DISCUSSION

This study confirms that sea-run Baltic salmon that produced offspring that later developed M74 have reduced tissue concentrations of thiamine. The family groups that developed M74 all had low thiamine concentrations, often 50% below those of healthy salmon family groups. The results of this study indicate that brown trout may also be affected by an M74-like reproduction disorder that is associated with low thiamine concentrations and several symptoms similar to those observed for M74 in Baltic salmon. However, the relatively limited evidence of reproductive disturbances in brown trout and the small sample size are not sufficient to provide conclusive evidence for the presence of thiamine-deficiency-related early-life stage mortalities in brown trout. The hepatic thiamine concentrations in healthy sea-run salmon and brown trout were similar, while egg thiamine concentrations of healthy brown trout were considerably higher than those of healthy Baltic salmon. The Baltic cod did not show ovarian thiamine concentrations. On the contrary,

they had the highest thiamine concentrations of all species measured in this study. The hepatic thiamine content in cod showed large variances between samplings, and also between females and males where the latter had a considerably higher hepatic thiamine content. A possible explanation for the lower hepatic thiamine concentrations in the females may be the transfer of thiamine to the gonads during spawning.

This study also revealed that variation in the time of onset of M74 in Baltic salmon correlates to the thiamine concentration of eyed eggs. The reduction in embryo thiamine concentration during progressive development, which previously has been shown to be similar in viable as well as in M74 offspring (5, 7), is probably of less importance for development of M74 than the initially deposited oocyte thiamine concentration. When combining these findings with the significantly lower thiamine concentrations in the ovaries and white muscle of female salmon whose progeny develop M74, it is evident that the M74 syndrome is caused by an insufficient maternal deposit of thiamine to the ovaries. The wiggling behavior seen in adult spawning salmon is also associated with reduced concentrations of hepatic as well as ovarian thiamine content, and may well be seen as the most severe form of M74-related thiamine deficiency that affects adult Baltic salmon. Also, the 3 family groups of salmon from Dalälven in 1998 (Fig. 3b), which had the highest thiamine concentrations within the groups that developed M74 in that year, did not show 100% M74, suggesting that development of M74 is dose-dependent and correlated to the initial thiamine concentrations of the progeny.

In the Laurentian Great Lakes of North America, a considerably larger number of salmonid species are affected by thiamine deficiency-related early-life stage mortalities in syndromes designated EMS (26, 27, 35) and Cayuga Syndrome (23–25). Eggs of lake trout (*Salvelinus namaycush*), coho salmon (*Oncorhynchus kisutch*), chinook salmon (*Oncorhynchus tshawytscha*), steelhead trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*), from lakes with documented EMS, had egg thiamine concentrations ranging between 1.2 and 4.3 nmol g⁻¹ (27). By comparison Baltic salmon eyed-eggs that developed M74 in this study had mean thiamine concentrations that ranged between 0.19 and 0.62 nmol g⁻¹, while those of normally developing eyed eggs ranged between 1.6 and 2.5 nmol g⁻¹. The latter concentrations are in the same range as for the eyed eggs afflicted by EMS. Atlantic salmon affected by Cayuga Syndrome have thiamine concentrations of a range similar to those found in Baltic salmon (5, 25). The requirement for thiamine seems to differ between different fish species and the variation in thiamine concentrations between salmonids affected by EMS, Atlantic salmon with Cayuga Syndrome and Baltic salmon with M74 appears to confirm this.

The thiamine concentrations in tissues from farmed Baltic salmon in this study were considerably higher than in sea-run individuals. The concentrations were 2–4 times higher in white muscle, and hepatic content of farmed salmon may indicate that sea-run salmon do not absorb and store sufficiently high quantities of thiamine. This may indicate that Baltic salmon consume prey species poor in thiamine, or that other mechanisms deplete the stored thiamine. Thiaminase I has been suggested to be the cause of Cayuga Syndrome and EMS, and it may also be a contributing factor for the development of M74, however, more research in this field is necessary.

The results of this study show that there are species differences in thiamine concentrations and that Baltic salmon with M74 have the lowest thiamine concentrations of all studied species. The preliminary indications of M74-like reproduction disorders in Baltic brown trout justify further investigations. Baltic cod eggs showed relatively high thiamine content but further studies are necessary to determine potential threshold levels for thiamine in Baltic cod.

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