M74 syndrome and thiamine in salmon broodfish and offspring

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The relationship was examined between the occurrence of M74 syndrome (abnormally high yolk-sac fry mortality in the Baltic salmon) and the thiamine (B_1 vitamin) concentration in the eggs, liver and white muscle of female salmon broodfish and in the fry. Newly stripped eggs with total thiamine concentrations of ca. 0.35 mg kg^{-1} or lower were shown to develop into M74 offspring. M74 syndrome was not found to exist in the Teno river Atlantic salmon or in the Daugava river Baltic salmon, but was prevalent in 1994–1996 in the Finnish rivers flowing into the Gulf of Bothnia. This is thought to be a result of the low reserves of thiamine in the broodfish during maturation in these rivers. It was shown that sexual maturation of female salmon broodfish entails the transport of a large amount of thiamine to the ovaries. The total thiamine content of the liver was only a fraction of that needed in the eggs, but white muscle tissue may act as a reserve for this purpose.

Introduction

In 1992–1997, abnormally high mortality (up to 90%) was observed in Finnish hatcheries among hatchery-reared yolk-sac fry of the Baltic salmon (the Baltic group of *Salmo salar* L.) originating from feral broodfish from the northern Gulf of Bothnia (Statistics of the Lautiosaari State Fish Hatchery, Keminmaa, Finland, unpubl. data 1997). Mortality at the same developmental stage has also been observed since 1974 in Sweden, where the disease was named M74 syndrome (Norrgren *et al*. 1993) referring to the Swedish word *miljöbetingad*, meaning environmentally-caused, and the first year of its recognition there. This syndrome (later M74) has not

been known to occur in hatchery-reared yolk-sac fry of the wild Atlantic salmon of the Teno river (P. Heinimaa, Inari Aquaculture, Inari, Finland, pers. comm. 1997) or the feral Baltic salmon of the Daugava river (Karlsson *et al.* 1996). Jokikokko *et al.* (1995) reported M74 to be the obvious reason for the rapid decrease in natural parr production found in 1992–1995 in the Simo and Tornio rivers, which have wild populations of the Baltic salmon.

Thiamine is an essential nutrient in fish (*see* Halver 1989, and Steffens 1989) and is required in its pyrophosphate form to introduce pyruvic acid from either glycolysis or amino acids into the citric acid cycle via acetyl CoA. Thiamine pyrophosphate is also a coenzyme for transketolase in the pentose pathway of hexose metabolism. Furthermore, thiamine pyrophosphate takes part in decarboxylation during the synthesis of the neurotransmitter acetylcholine, which carries a nerve impulse from one nerve cell to the next (*see* Reed 1980). Diets deficient in thiamine have for a long time been known to cause nervous disorders, poor appetite and growth and increased sensitivity to shock from light flashes in growing salmonids (*see* Halver 1989). Fisher *et al.* (1996) reported thiamine deficiency to be the cause of a mortality syndrome affecting the landlocked Atlantic salmon fry in the USA, and thiamine has subsequently been shown to be effective in the treatment of the Baltic salmon yolk-sac fry showing signs of M74 syndrome (Bylund and Lerche 1995, Amcoff *et al.* 1996). In the Great Lakes of North America, a thiamine deficiency syndrome called EMS (**E**arly **M**ortality **S**yndrome) has been reported in several salmonid species other than Atlantic salmon (Fitzsimons *et al.* 1999). Koski *et al.* (1996) showed thiamine hydrochloride to have a dose-dependent effect on the occurence of M74, which further emphasised its role in the aetiology of this syndrome. In addition to thiamine and toxicological factors (Norrgren *et al.* 1993, Vuorinen *et al.* 1997), differences between stocks in their feeding migration (Karlsson *et al.* 1996), possible annual differences in prey fish composition (Anon. 1994) and levels of astaxanthine (Lignell 1995, Börjeson *et al.* 1995) may also affect the occurrence of M74.

In this study, total thiamine levels were measured in the tissues of female salmon broodfish, newly-stripped eggs and yolk-sac fry originating from the Teno (Arctic Ocean), Kemi, Simo and Oulu rivers (Finnish side of the Gulf of Bothnia) and also the Daugava river (Latvia, Baltic proper). The aim was to identify a possible threshold concentration of thiamine in the eggs and fry at or below which the offspring would develop M74. The concentrations and total thiamine contents in the tissues of the broodfish were compared between locations where M74 does and does not exist. A feeding trial was conducted with farmed Baltic salmon broodfish to clarify the effect of the amount of thiamine in the diet on levels recorded in the tissues.

Material and methods

The fish and their husbandry

The sampling of salmon broodfish in this study is summarized in Table 1. The locations where the material was sampled are shown in Fig. 1. Farmed broodfish (location A) had been fed ad libitum (excluding the warmest period of the summer) until two weeks before stripping. Only a few fish still had a small amount of faeces in the rectum. The farmed broodfish were of the Ii river strain of Baltic salmon from the stock of Laukaa Aquaculture. Fish from other locations were known to be either wild or feral or both (Table 1). The basis of this information was the presence of a spawning wild population and the stocking of smolts in the location in question. The fishing gear comprised a drift net (location G), gill net (location B), hoop net (location E), seine net (location C) and trap net (locations D and F). The gastrointestinal tract was found to be empty in all dissected broodfish caught at the same time as the material from locations C–E, whereas the fish from location G had stomachs of variable fullness.

The farmed broodfish were fed from May to October 1995 on an artificial feed of the following composition (per kg of semi-moist feed): 350 g water, 440 g fish meal (Norse-LT 94, Norsimark, Helsinki, Finland, protein content 72%), 80 g bleached fish oil (Epax 3000 TG, Pronova Biocare, Bergen, Norway), 119 g wheat

Table 1. Data on the female broodfish salmon samples.

Location and the type of life cycle*	Year when the females were caught	Number of females examined	Stock and stage of the adult female when sampled	Location where the fish were caught	Dates of sampling	Weeks from capture to sampling	Mean GSI (%) (95% confidence interval)
Farmed fish							
A	1995	32	Baltic salmon, spawning broodfish	Laukaa Aquaculture	30–31 Oct.	–[1]	NA
Wild or feral fish							
B (w)	1994	12	Atlantic salmon, spawning broodfish	Lower part of Teno river	9–10 Oct.	6–7	20.0 (14.5–27.0)
	1995	5			2 Oct.	5–7	21.9 (16.0–25.5)
	1996	5			8 Oct.	6–7	16.1 (11.1–23.8)
C (f)	1995	7	Baltic salmon, spawning broodfish	Lower part of Kemi river	16–17 Oct.	17–18	19.0 (16.3–21.3)[3]
	1996	6			9–10 Oct.	14–17	16.4 (15.4–17.0)[3]
D (f/w)	1994	6	Baltic salmon, spawning broodfish	Sea in front of the mouth of Simo river	18–19 Oct.	15–20	NA
	1995	13			23 Oct.	16–19	NA
	1996	10			15–16 Oct.	18–19	18.3 (16.6–23.1)[3]
E (f)	1996	8	Baltic salmon, spawning broodfish	Lower part of Oulu river	22 Oct.	0–2	NA
F (f)	1996	10	Baltic salmon, spawning broodfish	Sea in front of the mouth of Daugava river	6–8 Nov.	0–6	NA
G (f/w)	1995	10	Baltic salmon, migratory adult females	Main basin of the Baltic Sea (ca. 58°23´–59°21´N, 19°30–38´E)	19–22 May	0[2]	0.65 (0.26–1.57)[2]

* The first letter refers to location in Fig. 1. The letter in brackets refers to the type of the life cycle of the broodfish of the location (w: wild — the location was not stocked; f: feral — the location was stocked, no wild population present; f/w: both wild and feral existed in the location).

[1] The fish were in a freshwater earth pond.

[2] Sampled, when the fish were caught.

[3] Not GSI, but 'GSI', *see* Materials and methods.
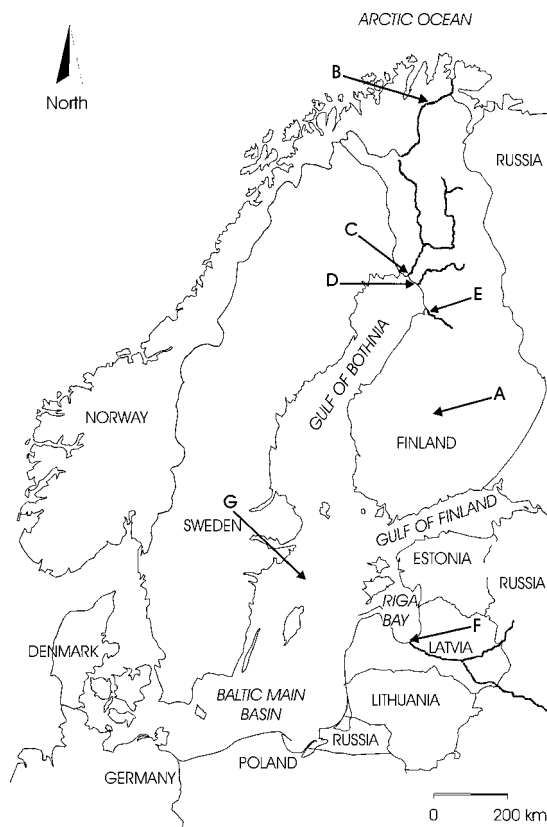
NA = Not analysed.

Fig. 1. Sites at which the broodfish studied here were caught. The capital letters refer to the first column of Table 1.

starch and 7 g guar gum. Vitamin premixes (Sareko, Finnewos Aqua, Finland) without thiamine or astaxanthine at a concentration of 4.5 g kg^{-1} feed and the same premixes with thiamine (10 mg thiamine/kg feed) and astaxanthine (Carophyl-Pink 8%, La Roche, Switzerland, 50 mg astaxanthine/kg feed) were added to the diet and represented normal and high thiamine levels, respectively. The feeds were frozen (–20 °C) and the amount required was thawed out daily. The following main characteristics of the feed were calculated on the basis of the certified ingredients (calculations according to Ruohonen and Vielma 1994): moisture 40%, protein 37%, lipids 11%, carbohydrates 12%, digestible energy 12.5 MJ mg^{-1}.

After collection, all broodfish were held prior to stripping in ordinary broodfish tanks or pens without feeding (Table 1). Within one

week of capture, the broodfish were measured for total length and weight and marked with a Carlin tag at locations B–D (Table 1). All fish were anaesthetized or stunned prior to stripping and the roe of each female was fertilized with the milt of 1–3 males. Eggs were collected for thiamine analysis from stripped roe before fertilization. Liver and muscle samples were taken within 15 minutes of stunning. Egg and tissue samples of about 5 g were placed in polypropylene tubes, frozen in liquid nitrogen and stored at –70 °C until chemical analysis, except at location F, where samples were kept on ice for 2–8 hours prior to freezing in liquid nitrogen. The total weight of each broodfish and the weight of the liver without the gallbladder were measured to the nearest gramme at the time of sampling. The weight of the eggs obtained by stripping and those left in the body cavity were measured for each female at location B, but only the weight of the eggs obtained by stripping was measured at locations C–D while the total weight of the gonads was measured for females at location G. The gonadosomatic index [GSI, Wootton (1990): GSI (wet mass, %) = 100 (wet mass of ovary or eggs at stripping (g)/total body mass (g))] was calculated for females from locations B and F. At locations C–D a separate 'GSI' was calculated as the ratio of the weight of the eggs to the total female weight, but the weight of the eggs obtained at stripping was used instead of the total mass of eggs. This 'GSI' was only determined for fish stripped once (Kemi river in 1995–1996 and Simo in 1996).

The fertilized ova and yolk-sac fry were incubated in the water of their respective farms and dead eggs were picked out where necessary. Samples of about 5 g of yolk-sac fry per female were stored in a similar manner to the egg and tissue samples at the age of 320–370 day-degrees after fertilization. All fish farms in this study used local surface water except location B, where untreated bore hole water was used. At some farms (locations A and F), standard malachite green baths were used to prevent Saprolegnia infection in the eggs. During the early yolk-sac period, 100–200 healthy yolk-sac fry were placed in plastic (location F) or aluminium trays (locations C and D). At the farm incubating the eggs from the Teno river the whole progeny of

the females was followed in a similar way. Fry were observed daily and dead individuals counted and removed every 1–3 days. Cumulative mortality from hatching until the time of the first feeding was determined. At farms incubating eggs from locations A and E, eggs from different females were combined so that female-specific mortality of the yolk-sac fry was not recorded as it was at the other farms.

Analogous microbiological and pathological studies to those presented in Koski *et al*. (1999) were performed with negative results on the broodfish and fry to exclude infectious aetiologies as a cause of death. However, these studies were not carried out at locations A and F.

Thiamine analysis

Total thiamine (free thiamine and thiamine mono- and pyrophosphates) was quantified by high performance liquid chromatography (HPLC) (Olli-lainen *et al*. 1993) with enzymatic hydrolysis modified according to Hägg (1995). Details of the analysis are presented in Koski *et al*. (1999).

The detection limit for total thiamine in this system was estimated to be 15 µg kg^{-1} tissue. Intratest reproducibility (variation within one day), calculated as a coefficient of variation (CV%), was 4.0% for the egg samples and 4.6% for the liver samples, and recovery percentages were 61%–87% for the liver samples and 72%–100% for the egg and fry samples. The reported thiamine levels have not been adjusted for percentage recoveries. No decrease in total thiamine concentrations was detected in egg samples from a single salmon stored at either –25 or –70 °C and analysed over a 15-month period. The effect on thiamine concentrations of tissue sample storage on melting ice at location F was not tested, but the figures were included in the analysis as they were higher values than those for the other Baltic locations.

Statistics

The underlying assumptions for parametric tests were often invalid because the data were asymmetrical or not normally distributed, or because

of the irregularity of the variation, and nonparametric tests were therefore used. The Kruskal-Wallis test was used to test for site-to-site variation in thiamine concentrations and cumulative percentage mortality. If significant differences were found, the Mann-Whitney *U*-test after adjustment with the Bonferroni method (Sokal and Rohlf 1995) was performed to determine which populations differed significantly from the others. Spearman's rank correlation was used to test for associations between the thiamine concentrations in different tissues and the GSI in females and also between the thiamine concentrations in the eggs and yolk-sac fry. The *p*-values for the Spearman's rank correlation coefficients were obtained from Diem and Lentner (1971). Statistical analyses were carried out using the analytical software packages SPSS/PC+ (Norušis 1986) or Statistix for Windows (Analytical Software 1996).

Results

Thiamine in farmed broodfish

Total thiamine concentrations in the white muscle (Mann-Whitney *U*-test $W = 138.0$, $n = 31$) and newly stripped eggs ($W = 444.0$, $n = 46$) of the farmed females that had received unsupplemented feed were similar to those in wild salmon from the Teno river (Tables 2 and 3). Although the median concentrations of thiamine in the liver were of the same order of magnitude (3.8 mg kg^{-1} in farmed and 3.2 mg kg^{-1} in Teno river females), they differed statistically ($W = 422.5$, $n = 46$, $p < 0.05$).

Total thiamine concentrations were significantly higher in the blood, liver and newly stripped eggs of the farmed broodfish that had received a diet supplemented with thiamine and astaxanthine as compared with those on an unsupplemented diet (Table 2; Mann-Whitney *U*-test: $W = 207$ for blood, $W = 220$ for liver and $W = 228$ for egg samples, $n = 32$, $p < 0.001$ in all comparisons). Spearman rank correlation tests showed no significant feeding group specific linear correlations between thiamine concentrations in the liver and eggs (Fig. 2; $r_s \approx 0.0831$, $n = 24$, $p > 0.05$ in the group receiving the
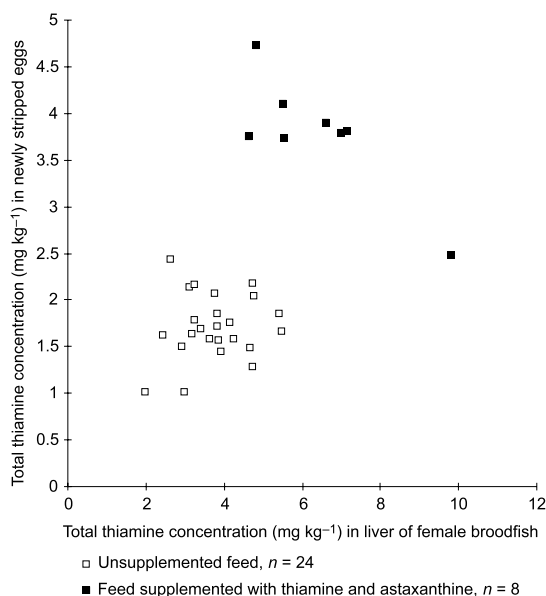
Fig. 2. Total thiamine concentrations (mg kg⁻¹) in the liver of the farmed female broodfish and the newly stripped eggs. The salmon were from location A (*see* Fig. 1).

Fig. 3. Scatterplot between total thiamine concentrations (mg kg⁻¹) in the newly stripped eggs and yolk-sac fry sampled prior to the appearance of clinical M74. Each square represents a sample of eggs and offspring from one female broodfish.

unsupplemented diet; $r_s \approx -0.4048$, $n = 8$, $p > 0.05$ in the other group).

M74 and thiamine concentrations in fry and eggs

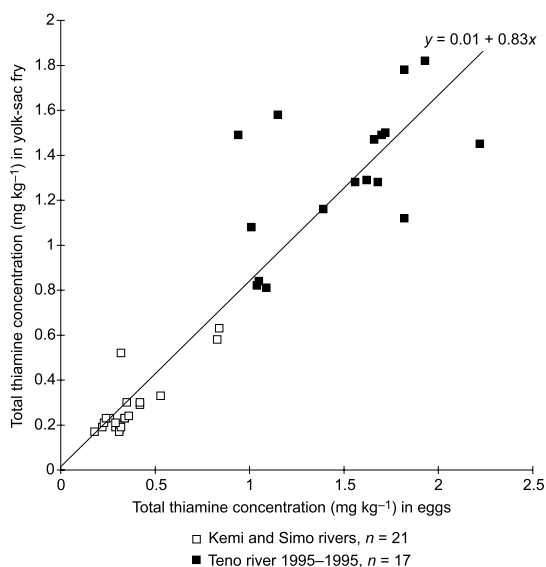A linear correlation existed between the total thiamine concentration in yolk-sac fry and that in newly stripped eggs (Fig. 3; Spearman rank correlation $r_s \approx 0.917$, $n = 38$, $p < 0.001$). The equation for a regression line fitted by the method of the least sum of squares to the data for locations B–D was:

$$y = 0.01 + 0.83x \tag{1}$$

where y = total thiamine concentration in the yolk-sac fry and x = total thiamine concentration in the newly stripped eggs.

The null hypothesis of identical annual cu-

Table 2. Total thiamine concentrations in blood, liver, white muscle and newly stripped eggs of farmed salmon on a non-supplemented feed and a feed supplemented with thiamine and astaxanthine at Laukaa Aquaculture (location A). The medians of the females on a supplemented and non-supplemented diet differ statistically (Mann-Whitney *U*-test, $p < 0.001$ in all comparisons). N = number of females examined.

	Females on a non-supplemented feed			Females on a feed supplemented with thiamine and astaxanthine		
	N	Median (range) (mg kg⁻¹)	Mean (mg kg⁻¹)	N	Median (range) (mg kg⁻¹)	Mean (mg kg⁻¹)
Blood	24	0.3 (0.1–0.8)	0.3	8	0.6 (0.4–1.0)	0.6
Liver	24	3.8 (2.0–5.5)	3.7	8	6.1 (4.6–9.8)	6.4
White muscle	9	2.1 (1.4–2.8)	2.2	NA	NA	NA
Eggs	24	1.7 (1.0–2.4)	1.7	8	3.8 (2.5–4.7)	3.8

NA = Not analysed.

Table 3. Total thiamine concentrations in the liver and white muscle of wild female broodfish and their newly stripped eggs and the cumulative mortality of the yolk-sac fry in different locations. N = number of females examined.

| Location[#] | Year[1] | N | Total thiamine concentration | | | | | | Cumulative mortality of the yolk-sac fry from hatching to first feeding | |
| | | | Female brood fish Liver | | White muscle | | Newly stripped eggs | | | |
			Median[2] (range) (mg kg^{-1})	Mean (mg kg^{-1})	Median[2] (range) (mg kg^{-1})	Mean (mg kg^{-1})	Median[2] (range) (mg kg^{-1})	Mean (mg kg^{-1})	Median[2] (range) (%)	Mean (%)
(B) Teno river	1994–96	22	3.2^a (2.1–4.6)	3.3	2.1^a (1.2–3.7)	2.3	1.6^{ad} (0.9–2.2)	1.5	1^a (0–16)	3
(C) Kemi river	1995	7	2.0^{bc} (1.7–2.1)	1.9	NA	NA	0.3^{be} (0.2–0.4)	0.3	100^{bc} (7–100)	87
(D) Simo river	1995	13	1.6^b (1.1–2.1)	1.7	0.5^b (0.4–0.8)	0.6	0.3^{bc} (0.2–0.8)	0.4	100^b (13–100)	71
(E) Oulu river	1996	8	1.6^b (1.1–2.1)	1.6	NA	NA	0.5^{bc} (0.2–1.7)	0.6	NA	NA
(F) Daugava river	1996	10	2.8^{ac} (1.9–4.1)	2.9	0.8^b (0.4–1.5)	0.8	1.2^{cd} (0.4–2.2)	1.2	9^c (1–53)	16
(G) Baltic Sea	1995	10	3.7^a (2.8–4.8)	3.8	0.5^b (0.2–1.1)	0.5	0.9^{ce} (0.2–1.2)*	0.8	NA	NA

[#] Letters in the brackets refer to the location in Fig. 1.

[1] Year, when the females were caught.

[2] Values with different letters in the columns are not identical (Mann-Whitney test after Bonferroni adjustment, $p < 0.01$). It can also be concluded that neither the median thiamine concentrations in eggs of Daugava and Simo river salmon, nor the median yolk-sac mortalities of Daugava and Kemi river salmon are identical (Mann-Whitney U-test after Bonferroni adjustment, $p < 0.05$).

* The values were measured from ovaries, not from newly stripped eggs.
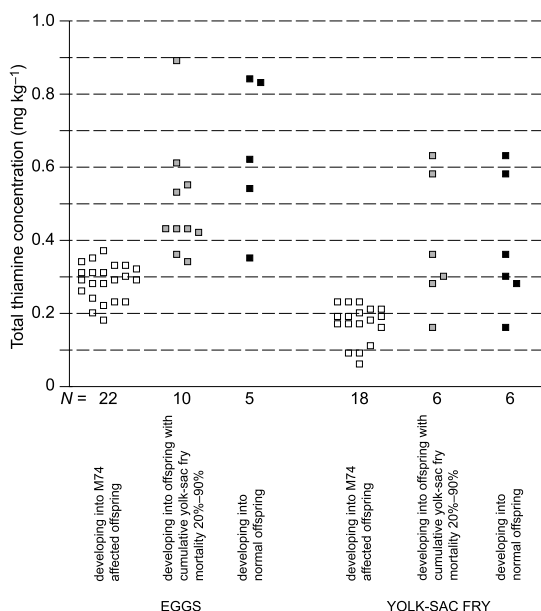
NA = Not analysed.

Fig. 4. Total thiamine concentrations (mg kg⁻¹) in the newly stripped eggs and yolk-sac fry prior to symptoms of M74 in offspring of Simo and Kemi river salmon in 1994–1996. Each square represents a sample of the offspring of one female brood fish (*N* = number of family groups examined). Criteria for the categories: eggs/yolk-sac fry developing into M74 offspring: cumulative yolk-sac mortality 90%–100% developing in a few days; eggs/yolk-sac fry developing into offspring with cumulative yolk-sac fry mortality of 20%–90%; eggs/yolk-sac fry into normal offspring: cumulative yolk-sac fry mortality under 20%.

mulative mortality in the years 1994–1996 could not be rejected on the basis of the material from the Kemi and Simo rivers (Kruskal-Wallis test: $p \approx 0.986$, $n = 68$). The data for these years was therefore pooled. Total thiamine concentrations were lower in the newly stripped eggs and yolk-sac fry of the salmon from the Kemi and Simo rivers in 1994–1996 which subsequently developed into offspring with M74 than in those not showing symptoms typical of M74 (Fig. 4). Not a single sample of eggs developing into offspring with M74 had a total thiamine concentration over 0.37 mg kg⁻¹, and none of those showing mortality ≤ 90% had a value under 0.34 mg kg⁻¹. The ranges of the yolk-sac fry thiamine concentrations overlapped somewhat more: the highest and lowest concentrations were 0.23 mg kg⁻¹ and 0.16 mg kg⁻¹, respectively (Fig. 4).

Yolk-sac fry mortality and thiamine concentrations in salmon caught from different locations

The median cumulative mortality of the yolk-sac fry from the early yolk-sac period to swim-up was 1% in the Teno river salmon, 9% in the Daugava river and 100% in the Kemi and Simo rivers (Table 3). The Bonferroni-adjusted Mann-Whitney test confirmed that mortality was significantly lower in the Daugava river than in the Simo ($p < 0.01$) and Kemi ($p < 0.05$) rivers, while mortality in the Teno river was significantly lower than at all other sites ($p < 0.01$).

Total thiamine concentrations in the liver, white muscle and newly stripped eggs of wild and feral female salmon broodfish varied significantly between the different locations from which they were sampled (Table 3; Kruskal-Wallis test: $p < 0.001$). In general, the highest concentrations of thiamine were in salmon from the Teno river followed by the Daugava river salmon, while the lowest concentrations were in the broodfish of the rivers entering the Gulf of Bothnia. The females of the Baltic proper (location G, Table 3) were on their feeding migration and their eggs had not yet begun to mature. These fish had higher concentrations of thiamine in the liver than the broodfish of the Gulf of Bothnia rivers (locations C–E; Fig. 1), but the median thiamine concentrations in the white muscle and the eggs did not differ significantly. The detailed results from different locations are presented in Table 3.

Thiamine budget in female salmon

The mean total thiamine content of the roe before stripping was calculated on the basis of the thiamine concentration in the eggs and the GSI of the female; for the Teno river salmon (1994–1996) it was ca. 0.29 mg kg⁻¹ live weight of fish and for the Kemi (1995–1996) and Simo (1996) river females it was ca. 0.07 mg kg⁻¹. The corresponding figure for female salmon from the Baltic proper at the end of the last spring of their feeding migration was ca. 0.0058 mg kg⁻¹ live weight of fish. The mean total thiamine content of the liver was ca. 0.031 mg kg⁻¹ live weight of

fish in the Teno river salmon (1994–1996), ca. 0.013 mg in the Kemi and Simo river salmon (1995) and ca. 0.045 mg in salmon at the end of their feeding migration in the Baltic proper. The Bonferroni-adjusted Mann-Whitney test confirmed that these results could not have originated from similar populations ($p < 0.001$ for all comparisons in this paragraph, except for the liver values between the Teno river and the Baltic proper where $p < 0.05$).

There was a positive linear correlation between the total thiamine content of the roe per kg broodfish and GSI in the Teno river (Spearman rank correlation coefficient $r_s \approx 0.6477$, $p < 0.01$; Fig. 5a), but not in salmon from the Kemi (1995–1996) or Simo (1996) rivers ($r_s \approx -0.1986$, $p > 0.05$; Fig. 5a). Thiamine concentrations in the liver and newly stripped eggs of female broodfish showed a significant positive linear correlation in the Daugava river ($r_s \approx 0.7622$, $p < 0.05$; Fig. 5b), but not in the Teno river ($r_s \approx 0.3077$, $p > 0.05$; Fig. 5b) or the Gulf of Bothnia rivers ($r_s \approx 0.0619$, $p > 0.05$; Fig. 5b). Strong positive linear correlations existed between thiamine concentrations in the eggs and white muscle of the female broodfish from the Simo river in 1995 ($r_s \approx 0.6611$, $p < 0.05$) and the Daugava river ($r_s \approx 0.8511$, $p < 0.01$). In the Teno river salmon, there seemed to be a year-to-year variation in this relationship. However, the low number of samples in 1995–1996 did not allow a proper statistical analysis of this matter.

Discussion

Thiamine concentrations in farmed broodfish

The total thiamine concentrations in the liver, newly stripped eggs and white muscle of the farmed broodfish which received unsupplemented feed agreed well with the values for the Teno river salmon (Table 3). The statistically significant difference between the median thiamine concentrations in the liver was probably not biologically significant, because the medians were of the same order of magnitude. Thiamine concentrations in the farmed broodfish which received unsupplemented feed were also compa-
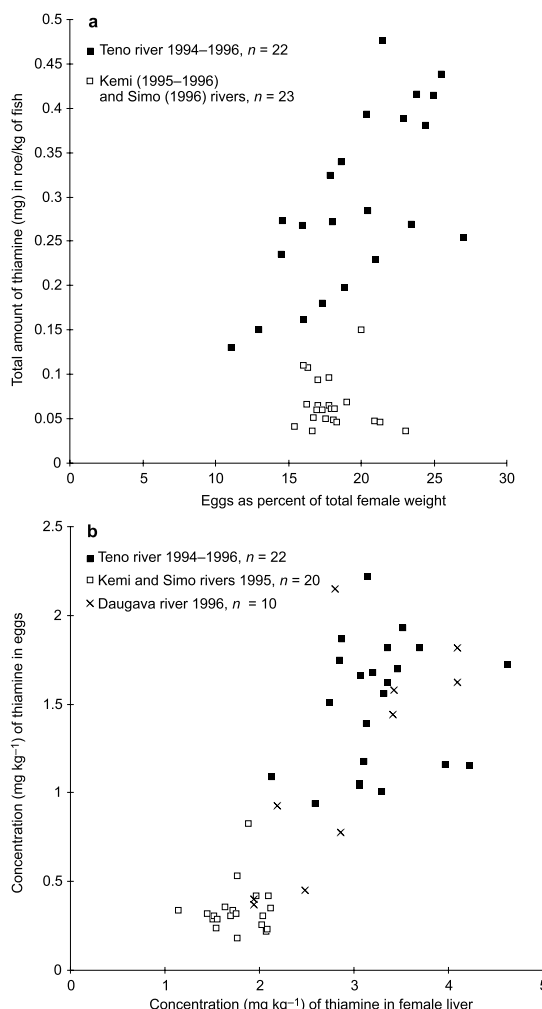

Fig. 5. Relations between thiamine concentrations or amounts in certain tissues of the female broodfish salmon. — **a**: Scatterplot between the weight of the eggs as a percentage of the total female weight and the total amount of thiamine in the roe per kg of fish. There is a significant positive linear correlation in the Teno river (Spearman rank correlation coefficient $r_s \approx 0.6477$, $p < 0.01$), but not in the Gulf of Bothnia river females ($r_s \approx -0.1986$, $p > 0.05$). — **b**: Scatterplot between the thiamine concentrations in the liver and eggs of the female broodfish. There is a positive linear correlation only in the Daugava river females (Spearman rank correlation coefficient $r_s \approx 0.7622$, $p < 0.05$), but not in the material from the Teno river ($r_s \approx 0.3077$, $p > 0.05$) or the Gulf of Bothnia rivers ($r_s \approx 0.0619$, $p > 0.05$).

rable with those from Baltic salmon broodfish fed on ordinary commercial dry salmon pellets. Our unpublished results for female broodfish of the Simo river salmon strain fed on commercial

dry feed (at location C; Fig. 1) show a mean total thiamine concentration of 3.6 mg kg^{-1} (range 3.0–4.6 mg kg^{-1}, n = 9) in the liver at the time of stripping. The higher thiamine concentrations found here in the blood, liver and eggs of the broodfish on thiamine-supplemented feed show that the supply of thiamine in the diet affects its concentrations in salmon tissues even when concentrations of thiamine in the feed exceed those needed to produce healthy eggs and yolk-sac fry. This is consistent with results presented by other authors for salmonids on thiamine-deficient and normal diets (Masumoto *et al.* 1987, *see also* Halver 1989, and Steffens 1989). When evaluating the concentrations of available thiamine in the food of migrating Baltic salmon, the effect of thiamine degrading substances must also be taken into consideration. The thiaminase activity of the prey fish reduces the available thiamine for the migrating Baltic salmon (Soivio and Hartikainen 1999).

The total thiamine concentration in the liver of female broodfish at spawning time was not found to be suitable for predicting the thiamine concentration in the newly stripped eggs, when the broodfish had been receiving an identical dietary supply of thiamine (Fig. 2). When comparing thiamine concentrations in different tissues of the farmed broodfish studied here with those in the wild fish, it must be remembered that the farmed fish were fed until about two weeks before stripping, whereas the wild spawning broodfish were not fed in the tanks or net-pens at locations B–F, and had therefore been without food for a period of from a few weeks to more than four months (locations C and D) before the stripping and sampling. The long fasting period before the spawning of wild salmon apparently places great demands on their metabolism with a short turnover time of substrates in broodfish. In view of the central function of thiamine in the metabolism and the results reviewed by Halver (1989) and Steffens (1989), this is probably also the case with thiamine. Our observation of higher liver thiamine concentrations in the farmed fish than in the Teno river wild salmon may indicate that the liver responds more rapidly than the white muscle tissue to the exhaustion of the thiamine supply during fasting.

M74 and thiamine concentrations in eggs and fry

The relationship between the total thiamine concentrations in the yolk-sac fry and newly stripped eggs of both the wild and feral salmon is a linear one, as seen in Fig. 3. This parallels the findings of Sato *et al.* (1987) of an approximately linear decrease in thiamine levels in rainbow trout relative to degree days from fertilization to first feeding. On the basis of Fig. 3 and the high Spearman rank correlation coefficient (ca. 0.917), we conclude that the total thiamine concentration in the eggs of feral or wild salmon is a good predictor of the total thiamine concentration in the yolk-sac fry before the appearance of M74. Whether this is also true for very high thiamine concentrations such as those obtained by injecting the female broodfish with thiamine (Koski *et al.* 1999) remains to be examined.

Amcoff *et al.* (1996) identified a threshold total thiamine concentration of ca. 0.110 mg kg^{-1} in newly fertilized ova at or below which the offspring of Baltic salmon in two Swedish rivers developed M74. Later, however, Amcoff *et al.* (1999) reported a mean threshold value (± SD) of 0.29 ± 0.07 mg kg^{-1} in newly fertilized ova of broodfish in the Lule river developing M74 offspring. The results obtained by different laboratories are not fully comparable, but our corresponding threshold of ca. 0.35 mg kg^{-1} in newly stripped eggs (Fig. 4) appears to be higher. Salmon eggs begin to swell because of the inflow of water during and after fertilization and this will lead to lower concentrations of thiamine in eggs sampled after fertilization than before. This effect may explain in part the difference between the results of Amcoff *et al.* (1996) and those of the present study. Koski *et al.* (1999) showed that the astaxanthine concentration may also have an effect on thiamine metabolism in the egg and the developing yolk-sac fry such that thresholds for M74 based on the thiamine concentration alone may be insufficient. Our results nevertheless indicate that total thiamine concentrations of ca. 0.35 mg kg^{-1} or lower in the newly stripped eggs will lead to M74 offspring. The mean whole body total thiamine concencentration reported by Amcoff

et al. (1996) in the yolk-sac fry developing into M74 offspring (0.0308 mg kg^{-1}) was lower than our lowest value (0.06 mg kg^{-1}; Fig. 4). Again, the later values of Amcoff *et al.* (1999) have been higher with the means of several groups of different years and rivers being ca. 0.03–0.20 mg kg^{-1}. Our results for yolk-sac fry correspond well with the higher mean values of Amcoff *et al.* (1999) and the values reported for a thiamine deficiency syndrome in landlocked Atlantic salmon in New York's Finger Lakes (Fisher *et al.* 1996). Although there is a need for intercalibration between laboratories, there may be a real difference in thiamine status in the yolk-sac fry of the salmon broodfish taken from the two Swedish and Finnish rivers of the Gulf of Bothnia. Wiggling behaviour in the broodfish was exceptional in our material, for instance, whereas this thiamine-related illness of adult fish (Larsson and Haux 1996, Amcoff *et al.* 1999) is not uncommon in Sweden (Börjeson *et al.* 1995).

Yolk-sac fry mortality and thiamine in wild and feral salmon at different locations

The majority of the growth during the feeding migration of the multi sea winter stock of the Teno river salmon occurs in the Norwegian Sea north of the Faeroe Islands (E. Niemelä pers. comm. 1997). Thus the prey fish of the Teno river salmon during their feeding migration are different from those of the Baltic salmon stocks. This is the most probable reason for the high concentrations of thiamine in the tissues of the female broodfish and in the eggs and yolk-sac fry of the Teno river salmon (Table 3 and Fig. 3) (Halver 1989). The low cumulative mortality of the yolk-sac fry at the farm incubating the offspring of the females at location B indicates that M74 syndrome does not occur in Teno river salmon. The range of thiamine concentrations in the newly stripped eggs at location B was also well above the threshold level for M74 presented in the preceeding paragraph (Table 3). The median cumulative mortality of the yolk-sac fry (9%) of salmon obtained from the Daugava river was substantially lower than that of the salmon from the Kemi and Simo rivers. In view of these

data together with the natural variability in the mortality of yolk-sac fry at Finnish salmon farms in the 1980s and the concentrations of thiamine in the eggs, we cannot state that M74 syndrome exists in Daugava river Baltic salmon.

The observation of lower total thiamine concentrations in the liver and eggs of female broodfish from the rivers flowing into the Gulf of Bothnia (locations C–E) in comparison with the other locations in the Baltic Sea catchment area (F–G) is interesting. The reason for the higher thiamine concentrations in the Daugava river (F) feral salmon than in salmon from the other Baltic rivers (C–E) may lie in a differing supply of thiamine during the feeding migration reflecting differences in the feeding areas or prey fish (Karlsson *et al.* 1996 and 1999). As the salmon of the Finnish Baltic rivers studied here (locations C–E) are known to migrate to the same feeding areas (Ikonen and Parmanne 1992, Salonen 1993, Salminen *et al.* 1994), differences in their thiamine supply during the feeding migration are thus improbable. According to Karlsson *et al.* (1999), the diet of Daugava salmon in the Bay of Riga was different from that of salmon migrating towards the Gulf of Bothnia rivers. The thiamine concentrations of the Daugava salmon in the Bay of Riga require further research before firm conclusions can be drawn from the differences relative to locations C–E. In addition to the composition of the diet, another possible dietary reason could be the duration of fasting prior to spawning or stripping. Salmon broodfish stop eating before they enter the spawning river (Kadri *et al.* 1995). As shown in Table 1, all the females in this study were caught at locations C and D at least 16 weeks before stripping, but less than 6 weeks beforehand at location F. If the fish caught at location F had been eating for longer than the females at locations C–D, this could have resulted in better reserves of thiamine at the beginning of maturation of the gonads. According to Karlsson *et al.* (1999), there are indications that the Daugava river salmon (F) feed later in the summer than salmon of the Gulf of Bothnia rivers. The total thiamine concentration in the eggs of the salmon in the Kemi and Simo rivers was low and the range was limited, whereas that

in the eggs of the salmon in the Oulu river (location E, where the fish were caught during the last two weeks before stripping) varied from similarly low levels up to levels comparable with those observed in the Daugava river (Table 3). The high concentrations could represent values for females that had entered the river mouth just prior to capture. The shorter period of fasting could also be responsible for the high thiamine values in the Daugava river. Although this hypothesis is plausible, it clearly needs to be confirmed by further research.

Thiamine budget in wild and feral female salmon

The females in the Baltic proper (Table 3; location G) were on their feeding migration and had similar concentrations of thiamine in their ovaries to those in the eggs of mature broodfish of the Baltic Sea (C–F). This might indicate that salmon do not build up a thiamine reserve in the ovary tissue before the onset of maturation.

The low total thiamine content of the liver of female salmon per kg live weight in comparison with that of the roe shows that the main reserves of thiamine for the eggs during the maturation cannot be stored in the liver. Assuming that the total white muscle mass was 60% of the total body weight (Smith 1982), the mean thiamine content in the white muscle tissue per kg live weight of fish was ca. 1.40 mg in the Teno river (1994–1996), ca. 0.33 mg in the Simo river (1995) and ca. 0.30 mg in the salmon from the Baltic proper at the end of the feeding migration. Brækkan (1959, 1969) reported concentrations of thiamine which would give a figure of ca. 0.78–1.20 mg per kg live fish for thiamine in the muscle tissue of the wild Norwegian Atlantic salmon. The mean total thiamine content of the liver per kg fish was ca. 11% and 19% of that in the roe in the Teno river, and the Kemi and Simo rivers salmon females, respectively. The mean total thiamine contents of the white muscle were almost five times as high as those in the roe in the Teno, Kemi and Simo river salmon. It is, thus, better to measure thiamine in the white muscle of the salmon broodfish rather than in the liver in order to monitor the thiamine

reserves of the fish. When the GSI is high, the total amount of thiamine in the roe also has to be taken into consideration when considering the thiamine budget.

The results underlying Fig. 5a can be explained by the difference in thiamine status between the female salmon of the Teno river and those of the rivers in the Baltic Sea catchment area. In the Teno river salmon, there was a positive linear correlation between the thiamine content of the newly stripped eggs and the GSI (Fig. 5a). This is probably because thiamine was not as lacking as in females of the rivers entering the Gulf of Bothnia. In these fish, the low total thiamine content of the roe was independent of the 'GSI' (Fig. 5a). The GSI and 'GSI' values obtained (Table 1) correspond well with the gonadosomatic indices reported for spawning Atlantic salmon in Norway and Canada (Jonsson *et al.* 1991 and 1997, Fleming 1996). Figure 5b indicates that the concentration of total thiamine in the liver of the female broodfish is not always a good basis for predicting the concentrations in the eggs and the future offspring. There is positive linear correlation only in the middle part of the scatterplot (the values for the Daugava river salmon females) while the values of the Gulf of Bothnia river salmon females represent the lag phase, and those of the Teno river salmon females the stationary phase of an S-curve (Fig. 5b). This could also explain the finding by Amcoff *et al.* (1996) of a lack of correlation between the thiamine concentrations in the female liver and eggs. Further research is required to determine whether muscle concentrations could be used for such a prognosis in the Baltic salmon. Although we regard the above suggestions to explain Fig. 5a and b, pharmacokinetics or studies with labelled thiamine are necessary to be more certain about the primary source and translocation kinetics of thiamine in female broodfish.

Vuorinen *et al.* (1997) attributed the low thiamine concentrations in the Baltic salmon eggs and yolk-sac fry to the effects of dioxin-like organochlorines. We consider that much of the variation in the prevalence of M74 and in the thiamine concentration in different salmon populations can be explained by variations in the dietary supply of thiamine. Analysis of both

toxins and thiamine (and astaxanthine) in the same Baltic salmon would nevertheless provide much more information on the aetiology of M74 than research which concentrates only on toxins or on thiamine (or astaxanthine).

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