The thiamine deficiency syndrome M74, a reproductive disorder of Atlantic salmon (*Salmo salar*) feeding in the Baltic Sea, is related to the fat and thiamine content of prey fish

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This study clarifies how the diet of Baltic salmon leads to thiamine deficiency in eggs, and consequently to M74 mortality of yolk-sac fry. The main prey species, sprat (*Sprattus sprattus*) and herring (*Clupea harengus membras*), and their biomass in the Baltic Proper (BPr) and the Bothnian Sea, the two feeding grounds of salmon originating from the northern Gulf of Bothnia rivers, are compared. The thiamine concentration of both prey species is lowest in the youngest age groups. Because average fat content and energy density are greater in sprat than in herring, and greatest in youngest sprat, the supply of thiamine per unit energy is least in a diet containing many young sprat. Also, the greater is the supply of thiamine and fat from sprat in the southern BPr in the preceding year, the lower the concentration of thiamine in salmon eggs. Thiamine deficiency in eggs results from an unbalanced diet abundant in fatty prey fish, such as young sprat, from which the supply of thiamine is insufficient in proportion to the supply of energy and unsaturated fatty acids for salmon, which must undergo a long prespawning fasting period.

Keywords: Atlantic salmon, energy density, fat, herring, lipid, reproduction, sprat, thiamine.

Introduction

The M74 syndrome of Atlantic salmon (*Salmo salar*) feeding in the Baltic Sea (hereafter Baltic salmon or salmon) manifests as offspring mortality during the yolk-sac fry phase (Bengtsson *et al.*, 1999; Keinänen *et al.*, 2000). A deficiency of thiamine (vitamin B1) in eggs is the immediate cause of M74 mortality (Lundström *et al.*, 1999b; Vuorinen and Keinänen, 1999), and it can be prevented by thiamine treatment (Koski *et al.*, 1999), as for salmonines exposed to early mortality syndrome (EMS) in the North American Great Lakes (Honeyfield *et al.*, 1998; Fitzsimons *et al.*, 1999) and Atlantic salmon exposed to the Cayuga syndrome in the New York Finger Lakes (Fisher *et al.*, 1995).

Most salmon from stocks in rivers flowing into the Gulf of Bothnia (GoB) migrate to the southern Baltic Proper (BPr) to feed, but a small proportion remain to feed in the southern basin of the GoB, the Bothnian Sea (BS, Figure 1). The proportion depends on the strength of recruitment of herring (*Clupea harengus membras*) in the BS (Ikonen, 2006). The principal prey species of Baltic salmon are sprat (*Sprattus sprattus*) and herring. In the BPr, the relative proportion in the diet has varied, but in the 1990s, when the incidence of M74 was high, the dominant species was sprat, whereas in the BS the proportion of herring was >90% (Karlsson *et al.*, 1999a; Hansson *et al.*, 2001). Salmon most often feed on fish <15 cm long, and even salmon that have reached 60 cm only occasionally prey on fish >20 cm (Hansson *et al.*, 2001). Therefore, all age groups of sprat, but only younger age groups of herring, are of a size appropriate as prey for salmon (Mikkonen *et al.*, 2011).
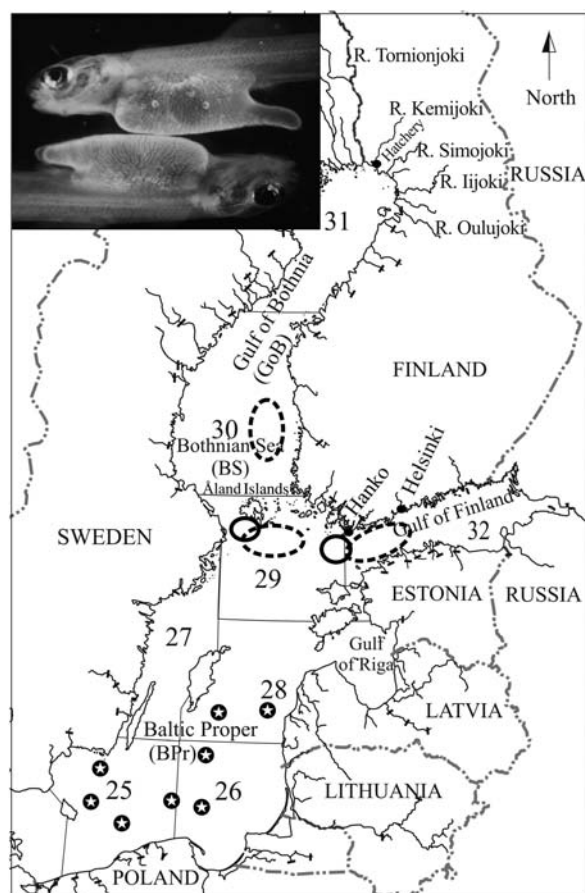
Figure 1. The Baltic Sea, showing the ICES SDs and the location of salmon spawning rivers. Sampling locations are indicated as follows: stars for fish caught in the southern BPr in March 1999, ellipses for fish from the northern BPr between October 1994 and January 1995 (Vuorinen *et al.*, 2002), and ellipses with a broken line for fish from the northern BPr, the BS, and the Gulf of Finland in autumn 2003 and spring 2004 (Vuorinen *et al.*, 2012). Insert: a yolk-sac fry with M74 symptoms (above), showing white-opacity-surrounded lipid droplets, and a healthy fry (below).

The M74 syndrome has been connected statistically to a large biomass and low individual weight of sprat and a large weight of salmon (Karlsson *et al.*, 1999a). A high condition factor (CF) among prespawning salmon predicts a high M74 incidence and together with a rapid growth rate has the strongest relationship with sprat biomass in the BPr (Mikkonen *et al.*, 2011). Based on congener profiles of dioxins and an increase in the concentration of coplanar PCBs in the muscle of salmon coincidentally with the outbreak of M74 at the start of the 1990s, salmon were concluded consistently to have been feeding to a large extent on sprat in which the concentrations of these organochlorines were also high in younger age groups with the greatest fat content (Vuorinen *et al.*, 2002). The thiamine concentration in sprat and small herring exceeded by several times the nutritional guidelines on growth of salmon, but it was lower in sprat than in herring (Vuorinen *et al.*, 2002).

As thiamine has a central role in energy metabolism, its nutritional requirement is determined by the energy density of the diet; it is estimated to be ~0.36 nmol kJ^{-1} for carnivorous fish (Woodward, 1994). The fat content of sprat is nearly twice that

of herring (Vuorinen *et al.*, 2002), and large quantities of fish fat in the diet increase muscle lipid concentration in salmonids and their susceptibility to lipid peroxidation (Alvarez *et al.*, 1998; Hemre and Sandnes, 1999). Abnormal lipid accumulation in the liver of M74 yolk-sac fry could result, for example, from endogenous feeding on rancid yolk or a deficiency of vitamin E (Lundström *et al.*, 1999a), and white opacities around lipid droplets in the yolk sac of fry (Figure 1, insert) displaying severe M74 may have arisen from the peroxidation of lipids (Keinänen *et al.*, 2000). A low concentration of carotenoids, especially astaxanthine, in eggs in M74 (Lundström *et al.*, 1999b; Pettersson and Lignell, 1999; Pickova *et al.*, 2003) and EMS (Palace *et al.*, 1998; Brown *et al.*, 2005) fish is seen as a pale egg colour (Börjeson *et al.*, 1999; Keinänen *et al.*, 2000). Deficiencies in thiamine and carotenoids in eggs might result from an insufficient dietary intake or elevated metabolic consumption in females, because both of these compounds function as antioxidants (Thomas, 1995; Gibson and Zhang, 2002). Variation in thiaminase activity in the prey fish of salmonids, herring in the Baltic Sea and alewife (*Alosa pseudoharengus*) in the Great Lakes and Finger Lakes in North America, has been inferred potentially to affect the extent of thiamine deficiency (Fisher *et al.*, 1996; Brown *et al.*, 2005; Fitzsimons *et al.*, 2005; Wistbacka and Bylund, 2008), but no clear evidence for this exists (Fitzsimons *et al.*, 2010; Mikkonen *et al.*, 2011). Instead, thiaminase activity in alewife of the Finger Lakes was negatively related to the lipid content of fish (Fitzsimons *et al.*, 2005).

Mikkonen *et al.* (2011) suggested that the M74 syndrome of Baltic salmon from GoB rivers is a nutritional distortion resulting from their feeding extensively on sprat. The aim of the present study was therefore to clarify the mechanisms that lead to thiamine deficiency in salmon eggs and hence to M74 mortality in yolk-sac fry.

Material and methods
Sampling and data for sprat and herring
Three datasets for sprat and herring were used in determining the average concentrations of nutrients for various age groups. The fish were sampled from different areas of the Baltic Sea in the years 1994/1995, 1999, and 2003/2004 (Table 1). The first dataset included data on the thiamine and fat concentrations of sprat and herring published by Vuorinen *et al.* (2002). The data were from fish collected off the Hanko Peninsula in ICES Subdivisions (SDs) 29 and 32 in the western Gulf of Finland and south of the Åland Islands (SD 29) from October 1994 to January 1995 (Figure 1). The sprat were 2–6, 8, 10, and 13 years old, and the herring 1–3 years old.

The second dataset was of thiamine and fat concentrations of sprat and herring collected from bottom trawls made by RV "Dana" during a groundfish survey in the southern BPr at eight sampling stations in SDs 25 (four stations), 26 (two stations), and 28 (two stations; Figure 1) in March 1999. Ten fish of both species per station were collected randomly and sealed individually in numbered polyethylene bags, then frozen (−20°C). In the laboratory, after weighing, measuring, and otolith removal, herring and sprat from each sampling station were sorted into large and small fish for homogenization as whole fish, with five fish in each pool. These pools were grouped based on total length, and the mean ages of sprat in the homogenates were 2.0–7.2 years and those of herring 2.0–8.0 years.

Table 1. Mean ($\pm$ s.e.) weight, total length, CF, and number of specimens (n1) sampled during 1994/1995 (in the northern BPr; Vuorinen et al., 2002), in 1999 (in the southern BPr), and in 2003 and 2004 (in the northern BPr, BS, and Gulf of Finland; Vuorinen et al., 2012), and the fat content (% in wet weight, ww), energy density (kJ g^{-1} ww), and total thiamine concentration (nmol g^{-1} ww) in respective homogenates (n2).

Sample	Weight (g)	Length (mm)	CF	n1	Fat (%)	Energy[a] (kJ g^{-1})	Thiamine (nmol g^{-1})	n2
Sprat, 1994–1995[b]	15.2 $\pm$ 0.1	140.7 $\pm$ 0.5	0.55 $\pm$ 0.00	208	10.8 $\pm$ 0.8	6.7 $\pm$ 0.3	6.69 $\pm$ 0.32	14
Herring, 1994–1995[b]	16.1 $\pm$ 0.1	139.6 $\pm$ 0.2	0.59 $\pm$ 0.00	146	7.6 $\pm$ 0.4	5.8 $\pm$ 0.2	8.62 $\pm$ 0.79	6
Sprat, 1999[c]	10.0 $\pm$ 0.4	120.3 $\pm$ 1.4	0.56 $\pm$ 0.01	80	9.2 $\pm$ 0.5	6.1 $\pm$ 0.2	7.71 $\pm$ 0.42	16
Herring, 1999[c]	30.0 $\pm$ 0.7	172.2 $\pm$ 1.3	0.58 $\pm$ 0.01	80	3.5 $\pm$ 0.2	4.3 $\pm$ 0.1	7.72 $\pm$ 0.72	16
Sprat, 2003–2004[d]	8.0 $\pm$ 0.1	112.0 $\pm$ 0.6	0.55 $\pm$ 0.00	544	9.3 $\pm$ 1.1	6.1 $\pm$ 0.4	5.00 $\pm$ 0.45	17
Herring, 2003–2004[d]	18.4 $\pm$ 0.4	140.2 $\pm$ 0.8	0.57 $\pm$ 0.00	1 410	5.7 $\pm$ 0.4	5.2 $\pm$ 0.1	4.42 $\pm$ 0.30	30

[a]Energy densities were measured in samples from 2003 and 2004 and calculated in samples from 1994, 1995, and 1999.
[b]October–January.
[c]March.
[d]October–March.

A third dataset consisted of sprat and herring collected from commercial midwater trawl catches from the northern BPr (SD 29), the BS (SD 30), and the Gulf of Finland (SD 32; Figure 1) during the last quarter of 2003 (autumn) and the first quarter of 2004 (spring). Fish were handled as described in Vuorinen et al. (2012). In the laboratory, age groups 1–3, 6, and 10 (=9–11) of herring and 1, 3 (=2 and 3), and 7 (6–8) of sprat were selected, and all herring and sprat belonging to the same group and season were pooled for homogenization and kept frozen (at −80°C). In this dataset, samples of 47 homogenates in all, because 1-year-old sprat were not caught in spring in the BS, were used to determine thiamine and fat concentrations and energy density.

The age of herring and sprat from the 1994–1995 and 1999 samples was determined from whole otoliths. For the samples from 2003 and 2004, otoliths were cut and the thin slices stained with a modification of the neutral red staining method. The CF of sprat and herring was calculated as in Mikkonen et al. (2011).

To determine the biomasses of sprat and herring in salmon feeding areas in the BPr (SDs 25, 26, and 28) and in the BS (SD 30) in the period 1976–2005, and for species comparison, i.e. sprat in the Baltic Sea (1974–2006) and herring in the BS (1976–2005), ICES Working Group (WG) results (ICES, 2006a, c) were utilized as in Mikkonen et al. (2011). Only herring <19 cm were included in biomass calculations. The oldest age group of herring included varied from year to year, from 1 to 5 years in SDs 25 and 26, from 2 to 8 years in SD 28, and from 4 to 8 years in the BS (SD 30; Mikkonen et al., 2011).

M74 data and salmon on their feeding migration

As described in Mikkonen et al. (2011), M74 monitoring data included annual mortality results from female-specific hatchery incubations of eggs and yolk-sac fry from 1985 on, and results from laboratory incubations for M74 symptoms and M74-related mortality from 1993 on (Keinänen et al., 2008) for salmon ascending the Rivers Simojoki, Tornionjoki, and Kemijoki (Figure 1). The spawning year (autumn) of salmon was used in connection with yolk-sac fry mortality (YSFM). For biochemical analysis, samples of ca. 100 g of unfertilized eggs with the ovarian fluid expelled were frozen when the eggs were stripped from the fish. Thiamine concentrations have been analysed in unfertilized eggs from M74-monitored salmon since the river ascendants of 1994.

To compare the annual growth and CF of salmon feeding in the BPr (SDs 24–29) with those feeding in the BS (SD 30), and the temporal relationship between the CF of salmon and YSFM, tag-recovery data from the Finnish Game and Fisheries Research Institute were used. The data consisted of salmon from north-eastern GoB rivers (Figure 1) tagged as smolts with Carlin tags after being trapped during their downward run or on release at the respective river mouth after hatchery rearing and caught at sea by driftnet or longline. For analysis, second sea-year feeding salmon (Salminen et al., 1994) caught between 1 September and 1 March, i.e. during their feeding migration, during the years 1979–2006 and having a CF of 0.60–2.80 were included. When years with <4 fish were omitted, data for 115 salmon from the BS and 717 from the BPr were available.

Biochemical analysis

The concentrations of free, i.e. unphosphorylated, thiamine (hereafter, THIAM), thiamine pyrophosphate (TPP), and thiamine monophosphate in homogenates of sprat and herring and in salmon eggs were determined as described in Vuorinen et al. (2002). Total thiamine (hereafter, thiamine) concentration was calculated as a sum of these three and was determined along with the diet of salmon. The thiamine status of salmon eggs was deemed to be indicated by the THIAM value, because that has been used in thiamine-based predictions of the incidence of M74 for the Baltic Salmon and Trout Assessment WG of ICES (ICES, 2006b), and it correlated more strongly with YSFM than the thiamine concentration of eggs, as also observed with EMS (Brown et al., 2005).

The total fat concentration in prey fish samples from 1994–1995 and 2003–2004 was derived from the procedure for analysing organohalogen concentrations at the National Institute for Health and Welfare (Kiviranta et al., 2004). The total fat concentration of herring and sprat samples in 1999 was determined by the Schmid–Bondzynski–Ratzlaff method (ISO 1735/IDF 5: 2004). The congruence of the two methods was ascertained by running 24 sprat and herring homogenates through both methods ($r^2 = 0.982$, $y = 1.01\,x - 0.53$, $p < 0.001$).

The energy density in samples from 2003 and 2004 was determined using an adiabatic bomb calorimeter (Automatic MK 200), according to the method of Raymond et al. (1957). From these data, coefficients of protein content, 0.157 for sprat and 0.176 for herring, were obtained. Applying these coefficients, the

energy densities in sprat and herring sampled in the years 1994–1995 and 1999 were calculated using the measured fat content and energy value of 37.26 and 16.73 kJ per 1 g of fat and protein, respectively (Kriketos *et al.*, 2000).

Calculations and statistical analysis

The body fat and thiamine concentrations, along with the CF and energy density of sprat and herring, were obtained from the three datasets. Linear regression was applied to determine the age relationships of the CF, the fat concentration, and energy density in sprat and herring. A second-order polynomial fit was used to obtain the age relationships for thiamine concentration and for thiamine per unit fat and energy. All age groups of herring were taken into account in determining the age relationships. From these relationships, the values of each parameter for different age groups of sprat and herring (herring total length <19 cm) were obtained. The annual thiamine and fat content and the energy density in salmon prey fish biomass in the main feeding areas of salmon originating from the northeastern rivers of the GoB, i.e. in the BPr (SDs 25, 26, and 28) and the BS (SD 30), were calculated for the period 1976–2005, taking into account the abundance of different age groups of both prey species (Mikkonen *et al.*, 2011). For species comparisons, similar calculations were performed too for units of total sprat biomass in the Baltic Sea (SDs 22–32; in 1974–2006) and for herring (<19 cm) biomass in the BS (SD 30; in 1976–2005).

One-way ANOVA with Tukey's *post hoc* test ($p < 0.05$) was applied to test the differences in thiamine and fat concentration and energy density, as well as in the thiamine content per unit fat and energy, between sprat and herring or within species between season, and between salmon prey biomasses in different areas.

Linear regression was applied in analysing the relationship between YSFM (1985–2006) and the energy content of salmon prey biomass in the BPr (SDs 25, 26, and 28 separately and combined) and in the BS (SD 30). Linear regressions or second-order polynomial fits, whichever yielded a better result, were calculated between the mean THIAM value in unfertilized eggs of salmon spawners and the thiamine, fat, and energy content and thiamine content per unit energy in the biomasses of sprat from the BPr (1993–2006) and herring from the BS (1993–2005). In analysing the relationships between parameters for sprat and herring stocks and those for M74-monitored salmon, a time-lag of 1 year was applied, i.e. data on salmon spawners were related to sprat and herring data from the previous year. This was because salmon already commence their spawning run in the BPr towards the northeastern rivers of the GoB in March and April, and feeding decreases during their migration, then ceases on arrival at the spawning river in midsummer (Karlsson *et al.*, 1999b; Ikonen, 2006), although they did feed throughout the previous year.

For the period 1988–1995, when the incidence of M74 was highest and sufficient data were available, the annual mean CF and growth of second sea-year feeding salmon were compared between the BPr and the BS by one-way ANOVA plus Tukey's *post hoc* test ($p < 0.05$). The CFs were treated as 3-year moving averages, because the numbers of tagged salmon recovered annually varied considerably. The annual growth was determined as in Mikkonen *et al.* (2011). The relationship between the CF of salmon caught from the BPr and the BS and YSFM in 1985–2005 and 1988–1995, respectively, was analysed by linear regression.

Statistical analysis was performed with the statistical software SAS (SAS Institute Inc.) version 9.2. For the calculations, percentage values were arcsin-transformed. Artwork was developed with the software package Origin (OriginLab) version 8.1.

Results

Fat, energy, and CF of prey species

The percentage of fat in both prey species was higher in autumn than in spring, and higher in sprat than in herring in both seasons, as well as when seasons were combined (Table 2). There was a positive linear relationship between the fat content and the CF in both species, but in sprat the increase in CF was more clearly related to the increase in fat percentage than it was in herring (Figure 2a). In sprat, the fat–CF relationship was significant in both autumn and spring, but in herring only in autumn.

The fat content was highest in young sprat, and it clearly decreased as a function of increasing age in autumn (Figure 2b). In herring, no such age–fat relationship was found, with seasons combined or tested separately. However, if herring >8 years old were omitted from the combined data, there was a decreasing trend with age ($y = 6.74 - 0.45\,x$, $r^2 = 0.206$, $p < 0.01$, $n = 46$).

Thiamine content in prey

Thiamine concentration changed curvilinearly with the age of both sprat and herring: thiamine concentration was lowest in the youngest age groups (and also in the oldest herring of length >19 cm, and hence not included as salmon prey) and greatest at 6–10 years in sprat and 3–7 years in herring (Figure 3a).

Table 2. Mean ($\pm$ s.e.) fat and total thiamine concentration and thiamine per unit fat and energy (E) in homogenates (n) of sprat and herring (<10 years old) sampled during 1994 and 1995 (in the northern BPr), in 1999 (in the southern BPr), and in 2003 and 2004 (in the northern BPr, BS, and Gulf of Finland).

Species, season	Fat (%)	Thiamine (nmol g^{-1})	Thiamine/fat (nmol g^{-1})	Thiamine/E (nmol kJ^{-1})	n
Sprat	9.7 $\pm$ 0.5 (1.9–17.3)*	6.4 $\pm$ 0.3 (2.5–10.5) n.s.	79 $\pm$ 7 (15–248)*	1.1 $\pm$ 0.1 (0.3–2.2)*	47
Herring	5.1 $\pm$ 0.3 (2.0–9.1)	6.2 $\pm$ 0.4 (2.5–12.2)	147 $\pm$ 14 (39–381)	1.3 $\pm$ 0.1 (0.5–2.9)	46
Sprat, autumn	11.2 $\pm$ 0.7[b] (5.4–17.3)*	6.2 $\pm$ 0.3[a] (2.5–8.6) n.s.	60 $\pm$ 5[a] (15–122)*	0.9 $\pm$ 0.1[a] (0.3–1.4) n.s.	23
Herring, autumn	6.9 $\pm$ 0.3[b] (4.3–9.1)	5.8 $\pm$ 0.6[a] (2.5–10.9)	86 $\pm$ 10[a] (39–187)	1.0 $\pm$ 0.1[a] (0.5–2.1)	24
Sprat, spring	8.3 $\pm$ 0.6[a] (1.9–14.1)*	6.7 $\pm$ 0.5[a] (2.6–10.5) n.s.	97 $\pm$ 12[b] (22–248)*	1.2 $\pm$ 0.1[b] (0.4–2.2) n.s.	28
Herring, spring	3.9 $\pm$ 0.3[a] (2.0–7.7)	6.5 $\pm$ 0.5[a] (2.9–12.2)	186 $\pm$ 18[b] (49–381)	1.5 $\pm$ 0.1[b] (0.7–2.9)	28

Ranges are given in parenthesis and significances between species are indicated on rows as an asterisk ($p < 0.05$) or as n.s. (non-significant), and as a superscript with a different letter indicating a significant difference between seasons within species.
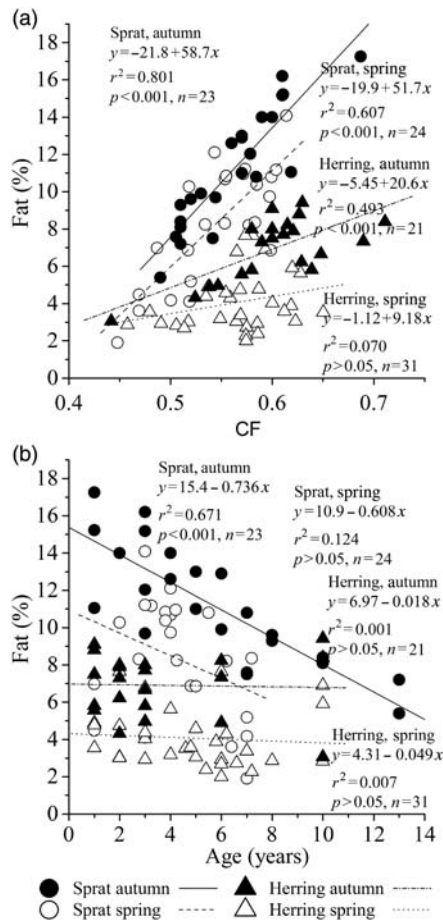
Figure 2. Fat content of sprat and herring (whole fish homogenates) in relation to (a) CF, and (b) age in autumn and spring, with the results of linear regression analysis (r^2 = coefficient of determination; n = number of homogenates).

Average thiamine concentrations did not differ between sprat and herring or between seasons in either species (Table 2).

There was no correlation between thiamine concentration and the percentage of fat in either species, which we attribute to considerable age-, season-, and area-dependent variation (Figure 3b). Thiamine concentration in proportion to fat content changed as a function of age in both species, being lowest in the youngest age groups (Figure 3c). Average thiamine concentration per unit fat was, however, higher in herring than in sprat, and in both species higher in spring than in autumn (Table 2).

A similar age relationship existed for the thiamine content per unit energy (Figure 3d), which was even lower in the youngest sprat than the guideline requirements for thiamine per unit energy in fish food (Figure 3d). The ratio of thiamine content to the unit of energy was lower in autumn than in spring in both species (Table 2), but the mean value was higher in herring than in sprat.

Comparison of sprat and herring as dietary components

For the whole sprat biomass of the Baltic Sea (1974–2006) and for the herring biomass of the BS (1976–2005), the concentration of thiamine had a negative linear relationship with fat concentration and energy density (Figure 4). However, the thiamine content per

unit energy or fat was lower in the sprat biomass of the Baltic Sea than in the herring biomass of the BS (Figure 4).

Mean annual thiamine content per unit of biomass of the salmon prey fish was higher in the BS than in the BPr areas SD 25, 26, or 28, and it did not differ in this respect (Figure 5a). On the contrary, fat content and energy density were both considerably higher in the southern BPr than in the BS, and the BPr differed in that values were lowest in the northernmost area, SD 28, and highest in SD 25 (Figure 5b and c). Thiamine content per unit fat (not shown) or energy (Figure 5d) was higher in salmon prey biomass in the BS than in the three BPr areas.

Salmon diet, growth, CF, and M74

In the BPr, the biomass of sprat increased from 7×10^8 kg in the late 1980s to 20×10^8 kg in the mid-1990s, when the increase was most pronounced in the two southernmost of the three SDs, i.e. SDs 25 and 26 (Figure 6a). At the same time, the biomass of herring decreased by ~19%, after having been at the same level or even greater than the sprat biomass in SDs 25, 26, and 28 from the late 1970s to the late 1980s. The decrease in herring biomass was most obvious in SDs 25 and 26. In the BS, the biomass of herring increased from 1.1×10^8 kg in the early 1980s to 4.3×10^8 kg in the mid-1990s (Figure 6a).

A clear positive linear relationship existed between the mean YSFM (1985–2006) and the energy content of salmon prey biomass in SDs 25, 26, and 25 + 26 (Figure 6b). The positive relationship between YSFM and the energy content of the biomass of salmon prey fish in SDs 25 + 26 + 28 was also significant, but not for SD 28 alone. A significant, but less clear, relationship was detected between YSFM and the energy content of herring biomass in the BS (Figure 6b).

The mean annual growth of second sea-year feeding salmon was higher ($p < 0.01$–0.001) in the BPr (range 25.0–28.0 cm year^{-1}, $n = 37$–260) than in the BS (20.5–24.7 cm year^{-1}, $n = 17$–34) from 1989 to 1992. The CF of second sea-year feeding salmon from 1988 to 1995 was likewise higher ($p < 0.001$) in the BPr than in the BS. The mean YSFM changed with the CF of salmon from the BPr (1985–2005), but not with the CF of salmon from the BS (1988–1995; Figure 7).

The THIAM value in eggs of salmon monitored for M74 was related negatively with the thiamine content in Baltic Sea sprat biomass (1993–2006), i.e. the higher the thiamine content in sprat biomass in the year before salmon spawning, the lower the mean THIAM value in the eggs of females ascending the rivers (Figure 8a), indicating a higher probability of M74. The THIAM value of eggs was similarly negatively related to the fat and energy content of sprat biomass in the Baltic Sea. The THIAM value of salmon eggs was positively related to the thiamine content per unit energy in total sprat biomass in the Baltic Sea (Figure 8a). There were no significant relationships in any of these parameters between the THIAM value of eggs and the biomass of herring in the BS (1993–2005), and contrary to sprat biomass, the relationship between thiamine content per unit energy with the THIAM value of eggs tended to be negative (Figure 8b).

Discussion

Fat and energy of prey species

The most pronounced difference between sprat and herring as food for salmon was the higher lipid content and energy density
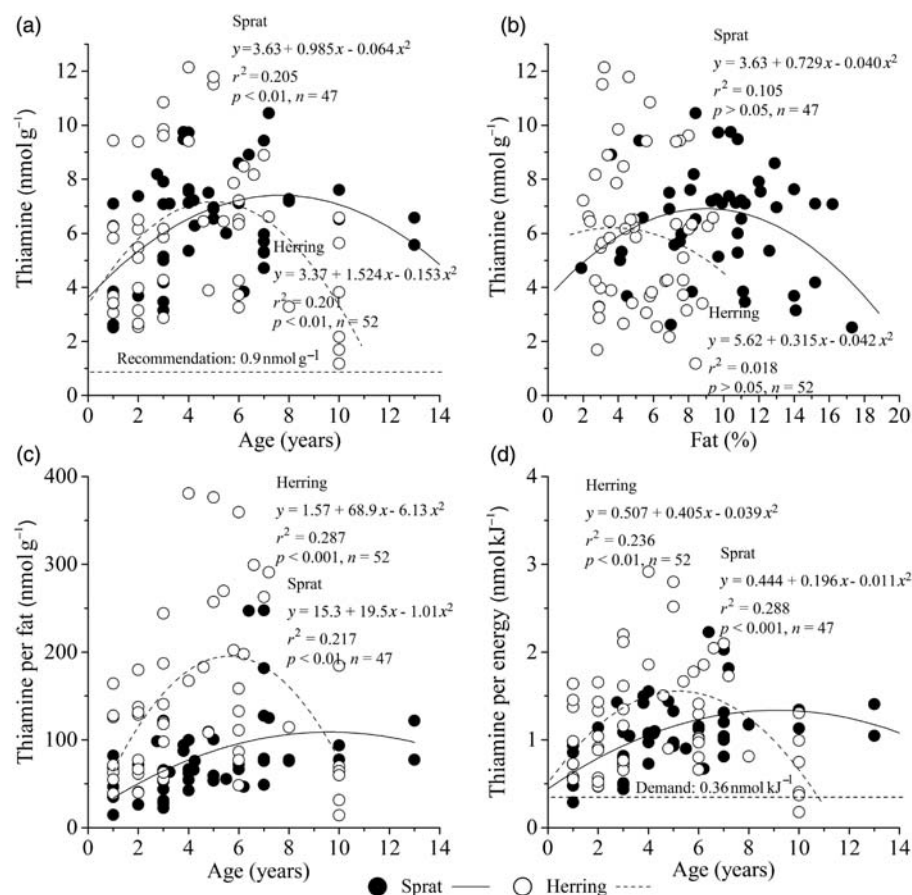
Figure 3. Total thiamine concentration of sprat and herring (whole fish homogenates) in relation to (a) age and (b) fat content, and the total thiamine content (c) per unit fat and (d) per unit energy in relation to the age of sprat and herring, and the results of non-linear analysis (r^2 = coefficient of determination; n = number of homogenates). The recommended thiamine concentration in feed and its requirement based on the food energy content, according to Woodward (1994), are given in (a) and (d), respectively.

of sprat. Sprat appear to accumulate lipids more readily than herring, especially in good conditions for growth such as those associated with more southern parts of the Baltic Sea. This was seen in the ratio of the fat content of sprat to that of herring, i.e. 1.7 (spring 2004) in the northern compared with 2.6 in the southern Baltic Sea (spring 1999). During 2003 and 2004, the fat content in autumn was 1.5–2.7 times greater in sprat and 1.2–1.5 times greater in herring than in spring, depending on area, which also indicates that the percentage of lipid can vary more in sprat than in herring. Simm *et al.* (2006) reported congruent differences in lipid content between autumn (11.5–15.7%) and spring (1.2–12.0%) sprat from the northeastern Baltic Sea in the years 2002–2004. Greatest lipid content in the youngest age groups of sprat, decreasing linearly with age, was also detectable in the data of Simm *et al.* (2006) and Pandelova *et al.* (2008), as in this study. In herring, the age relationship with fat content was not clear, and in the data of Pandelova *et al.* (2008), there was no relationship.

Contrary to salmon in GoB rivers, salmon from rivers flowing into the Gulf of Riga have not displayed M74 (Karlsson *et al.*, 1999b; ICES, 2006b). The main prey species of salmon feeding in the Gulf of Riga is herring, and the salmon diet there does not include sprat, though sprat was the dominant prey species in the BPr during the years 1995–1997 (Karlsson *et al.*, 1999a;

Hansson *et al.*, 2001). In the Gulf of Riga, in contrast to salmon feeding in the BPr or the BS, the proportion of alternative prey fish species, such as sandeels (*Ammodytes* spp.), perch (*Perca fluviatilis*), smelt (*Osmerus eperlanus*), and cod (*Gadus morhua*), can be considerable (Karlsson *et al.*, 1999a; Hansson *et al.*, 2001). The fat content of these fish species is less (0.8–8%; Ruus *et al.*, 1999; Pandelova *et al.*, 2008) than sprat, except perhaps smelt (for which the proportion in the diet is <4%). Three-spined stickleback (*Gasterosteus aculeatus*), the fat content of which is ~10% (Vuorinen *et al.*, 2012), are part of the salmon diet in all three sea areas.

The incidence of EMS and the Cayuga syndrome have also been associated with feeding on an unbalanced diet of alewife, following changes in its abundance and recruitment (Fisher *et al.*, 1996; Fitzsimons *et al.*, 1999, 2010; Brown *et al.*, 2005; Ketola *et al.*, 2009). In Lake Michigan, the diets of Chinook (*Oncorhynchus tshawytscha*) and coho salmon (*O. kisutch*), which have exhibited greater incidence of EMS than lake trout (*Salvelinus namaycush*; Fitzsimons *et al.*, 1999), were dominated by alewife when abundant, whereas the diet of lake trout was more diverse, including more rainbow smelt (*Osmerus mordax*), the second most common prey item (Brandt, 1986; Jude *et al.*, 1987). The fat content of alewife in Lake Michigan was 2–19%, depending on season, area, and body size, i.e. similar to sprat, whereas in
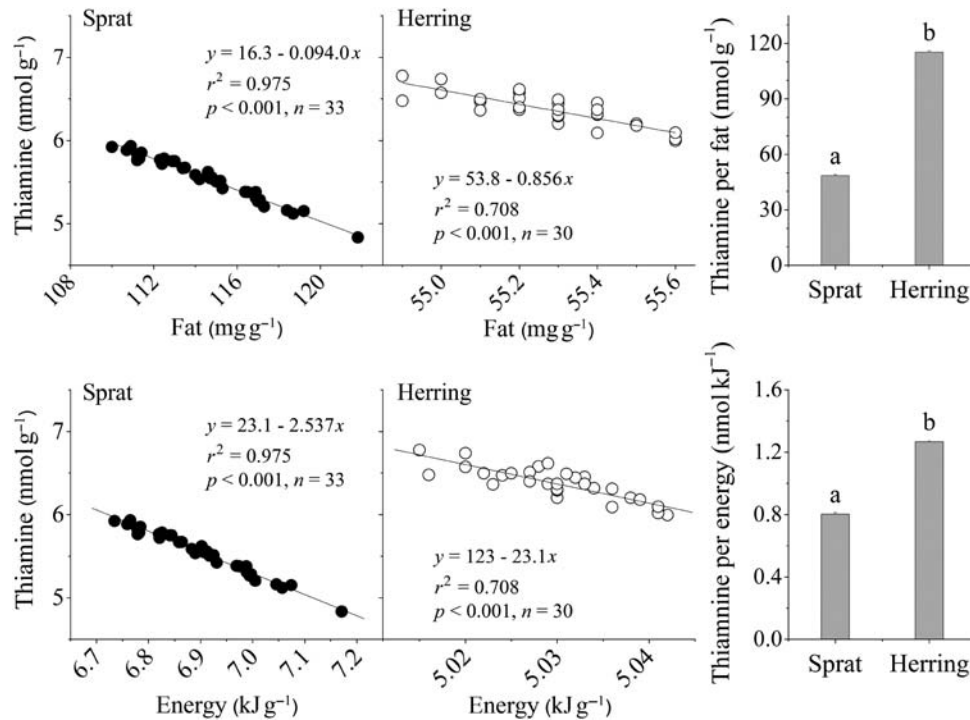
Figure 4. Annual mean total thiamine concentration in relation to fat content and energy density in the sprat biomass of the Baltic Sea (1974–2006) and in the herring (<19 cm) biomass of the BS (1976–2005), with the results of linear regression analysis (r^2 = coefficient of determination; n = number of years; note different scale for energy and fat between species), and as means ($\pm$ s.e.) of the annual values [columns with a different letter above them indicate a significant ($p < 0.05$) difference between sprat and herring]. Biomasses were derived using datasets from ICES (2006a, c) and from Mikkonen et al. (2011).
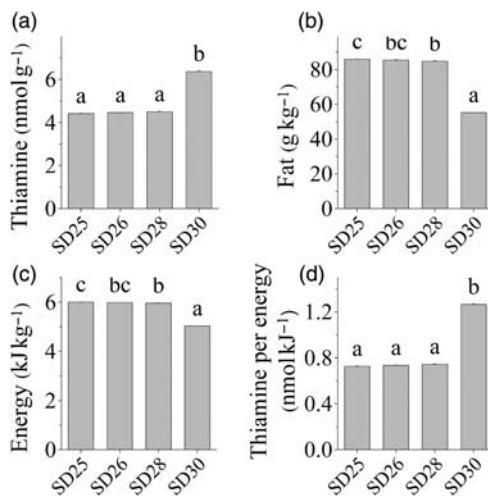

Figure 5. (a) Total thiamine and (b) fat concentration, (c) energy density, and (d) total thiamine content per unit energy of the total prey biomass (herring <19 cm plus all sprat) in ICES SDs 25, 26, and 28 in the BPr and in the BS (SD 30). The values are means ($\pm$ s.e.) of the years 1976–2005, and a different letter above the columns indicates a significant ($p < 0.05$) difference between SDs. Biomasses of herring and sprat are taken from Mikkonen et al. (2011).

rainbow smelt it was 1–6% (Madenjian et al., 2000). Much like in Baltic sprat in our study, and also in the data of Simm et al. (2006) and Pandelova et al. (2008), the percentage of fat in alewife has varied markedly over the years.

Because of greater variation in lipid concentration, the energy density varied more in sprat than in herring in the present study. The average energy density in alewife was also higher and varied more than in rainbow smelt (Rand et al., 1994). Similar to sprat, alewife appear to accumulate fat readily under favourable conditions; in Lake Ontario and Lake Michigan, the energy density of alewife varied more than twofold between the autumn peak and late spring and was also related to the age group (Rand et al., 1994). Compared with alewife, the average energy density in sprat was somewhat higher and in herring lower, whereas in both species it was higher than in rainbow smelt (Rand et al., 1994).

M74 and dietary fat and energy content
The higher CF of salmon feeding in the BPr than in the BS during the period with a high M74 incidence indicates that the M74 females originated from the BPr, because according to Mikkonen et al. (2011), a high CF of prespawning salmon is indicative of high YSFM. Salmon also grew faster in the BPr than in the BS during years with the greatest incidence of M74 in the early 1990s, similar to the findings reported for the 1980s by Salminen et al. (1994). A large body weight of spawning salmon was also associated with the incidence of M74 in general (Karlsson et al., 1999a), and intra-annually in years of relatively high M74 during the 1990s (Mikkonen et al., 2011). Similarly, large body weights of Atlantic salmon and lengths of coho salmon from Lake Michigan were related to low egg thiamine concentration, and therefore, greater levels of EMS (Wolgamood et al., 2005; Werner et al., 2006). On the other hand, the length and
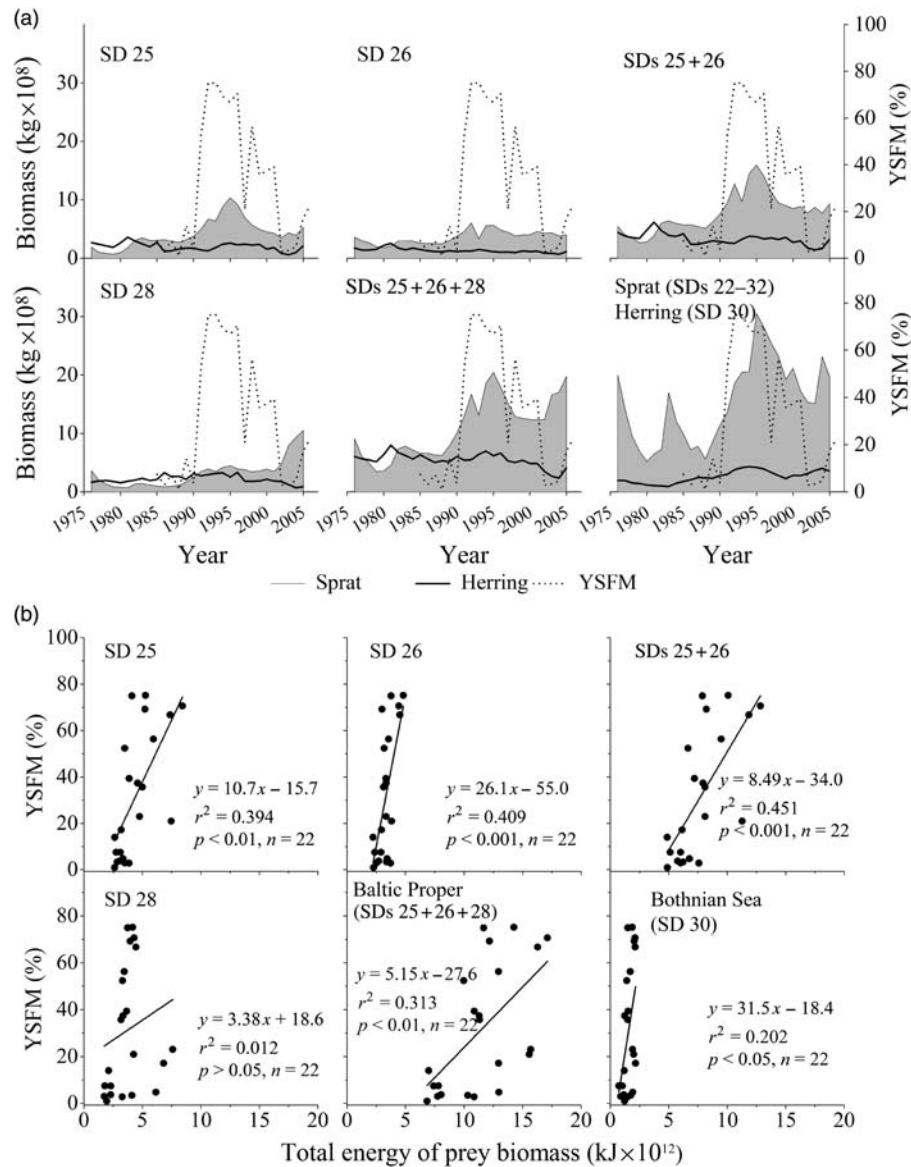
Figure 6. (a) Biomasses of sprat (grey area) and herring (thick line) in the BPr in ICES SDs 25, 26, and 28, total biomass of sprat in the Baltic Sea (SDs 22–32) and biomass of herring in the BS (SD 30) in 1976–2005, and the annual mean YSFM (dotted line) among offspring of salmon spawners from GoB rivers in the years 1985–2006. (b) YSFM in relation to the energy content of the salmon prey biomass of ICES SDs 25, 26, and 28 in the BPr and of the BS, with the results of linear regression analysis (r^2 = coefficient of determination; n = number of years). The biomasses of sprat and herring (<19 cm) are taken from Mikkonen *et al.* (2011) and derived using datasets from ICES (2006a, c).

growth rate of salmonines correlated positively with CF, energy content, and abundance of certain year classes or age groups of alewives in Lake Ontario (O'Gorman *et al.*, 1987; Ketola *et al.*, 2009). The incidence of M74 was most clearly related to the energy content of the prey biomass in the southernmost BPr, suggesting that salmon with offspring displaying M74 most probably fed there, because a lipid-rich diet increases the fat content of Atlantic salmon (Hemre and Sandnes, 1999).

A high sprat-to-herring ratio in prey biomass increases the fat supply to salmon. However, this supply is highest at times and in areas when and where young age groups of sprat dominate. In the early and mid-1990s, salmon had an abundant supply of food that consisted mainly of sprat and especially of young sprat in the BPr (ICES, 2006a). In contrast, from 1976 to the end of

the 1980s, when M74 mortalities were not reported as high, the proportion of herring in salmon prey biomass exceeded that of sprat, or these species were equally abundant in the BPr. Strong recruitment of sprat was evidently reflected in smaller sprat (age groups 0 and 1) in the salmon diet during the years 1994–1997 compared with the years 1959–1962 (Hansson *et al.*, 2001). During the 1990s, both the incidence of M74 and the CF of female salmon were at their lowest levels in 1997, coincident with a rapid decline in the size of the sprat stock. During the period 2003–2005, when the sprat stock was at its smallest since the start of the 1990s, M74 mortality was insignificant (Mikkonen *et al.*, 2011).

Correlation between YSFM and the energy content of prey biomass in the BS evidently only reflects the overlapping increase
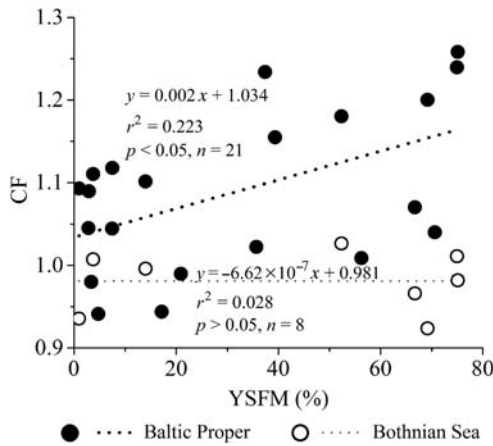
Figure 7. Relationship between CF (as a 3-year moving average) of second sea-year salmon caught annually from 1 September to 1 March in the Baltic Sea in ICES SDs 24–29 during the years 1985–2005 and in the BS during the years 1988–1995, and the annual mean YSFM among offspring of salmon spawners, and the results of linear regression analysis (r^2 = coefficient of determination; n = number of years). The number of salmon from the Baltic Sea was 6–382 and from the BS 4–83, and the annual number of M74-monitored females from the rivers flowing to the northeastern GoB was 13–84.

in herring biomass there with sprat biomass in the BPr (Mikkonen et al., 2011). The present study demonstrated that YSFM was related to the CF of salmon from the BPr, but not from the BS. Moreover, the growth rate and CF of prespawning salmon in the BPr were connected to the biomass of young age groups of sprat, in addition to total sprat biomass, but not to the biomass of young herring age groups in the BS (Mikkonen et al., 2011). The proportion of salmon foraging in the BS has been small (4–19%; Ikonen, 2006) compared with that of M74 salmon (54–92%) in years of high M74 incidence (1992–1996; ICES, 2006b). Therefore, feeding mainly on herring, with its thiaminase activity ca. tenfold higher than in sprat (Wistbacka and Bylund, 2008), does not appear to be a plausible explanation for a high incidence of M74.

The fat and energy content of salmon prey biomass in the BPr would have most probably been even higher than estimated if the data had also contained autumn samples from the southern BPr, when the fat and energy content were highest, and if sprat of age group 0 had been included in the calculations. Conversely, the leanest prey fish from the Gulf of Finland reduced the estimated fat and energy values. Sprat of age group 0 were not taken into account here, however, because only a small proportion of them, and not in every year, were included in available ICES WG results (ICES, 2006a, c). However, salmon feed on prey items shorter than 5 cm, and prey heavily on fish of length 5–10 cm (Hansson et al., 2001). Sprat aged 0 fall well within this length range during the last quarter of their hatching year. In November and December, their energy content was nearly as great as that of sprat aged 1 year in spring in the northwestern Baltic (Arrhenius, 1998) and was much higher than the energy content of sprat of age group 1 in spring in the Gulf of Finland.

Thiamine in prey species
Although the thiamine concentration in the combined data did not differ between species or seasons, in the autumn data from

1994 and 1995, the thiamine concentration in 2–13-year-old sprat (6.7 nmol g^{-1}) was lower than in 1–3-year-old herring (8.6 nmol g^{-1}; Vuorinen et al., 2002). Age-dependent variation was clear in both species, but notable within-age variation was not related to sampling area, season, or year. In alewife and rainbow smelt from Lake Michigan, the thiamine concentration varied more than in sprat or herring and was lowest in spring and highest in summer (Tillitt et al., 2005). However, the thiamine concentration did not correlate with the leanness (CF) of alewife from the Finger Lakes (Fitzsimons et al., 2005), which was also the case for sprat and herring in the present study. The maximum thiamine concentrations in herring were in age groups 3–7, but in sprat not until age groups 6–10 years. The ratio of older to young sprat evidently also increased after the mid-1990s following a rapid decrease in the recruitment of the species (ICES, 2006a), which was reflected in a temporal increase in the THIAM value in salmon eggs and a decline in M74 in 1997 (Mikkonen et al., 2011).

Supply of thiamine and M74
The decrease in THIAM values in salmon eggs in relation to the increase in energy and fat content in the sprat biomass of the BPr, along with the positive relationship between egg THIAM and the thiamine content per unit energy there, indicates that the M74 syndrome is associated with a limited supply of thiamine in proportion to the supply of fat specifically from the BPr. The absence of a relationship between the THIAM value in eggs and the same lipid-related parameters of the herring biomass in the BS is indicative of there being no cause–effect relationship with M74, at least in years with high M74 incidence. The physiological value of fat as fuel, i.e. its net energy value, is more than double that of protein (Kriketos et al., 2000), and the thiamine requirement depends on the energy density of food (Woodward, 1994). Contrary to demand, thiamine concentration decreased as a function of an increase in the fat content of herring biomass in the BS, and especially of sprat biomass in the Baltic Sea. This inverse relationship is clearly related to changes in the abundance of the youngest age groups of prey fish (ICES, 2006a). Considering the demand, the supply of thiamine for salmon is lowest at times and in areas with strong recruitment of sprat and herring and was at its least in the BPr during the outbreak of M74 at the start of the 1990s (Mikkonen et al., 2011). As thiamine concentration per unit fat or energy of prey fish biomass was least in the southern Baltic, salmon with a deficiency of thiamine in their eggs and a higher incidence of M74 among their offspring were most probably the ones that fed there, coincident with a large sprat biomass.

The average values of nutrients from spring and autumn were used to calculate the nutrient content of prey biomass by species, but the fat content in both prey species was greater in autumn than in spring, and the thiamine content per unit energy was consequently lower. If salmon feed more in autumn and early winter than in spring, and in mild winters in increasing numbers specifically on sprat (Karlsson et al., 1999a), the actual thiamine intake per unit energy in the southern Baltic would be even lower than estimated during the strong recruitment years of sprat at the start of the 1990s. YSFM peaked during those years, whereas the energy density of sprat, based on their CF (Mikkonen et al., 2011) was lowest only thereafter, in 1995 and 1996. Shortly thereafter, in 1997, the incidence of M74 decreased temporarily. In our average estimations, the thiamine
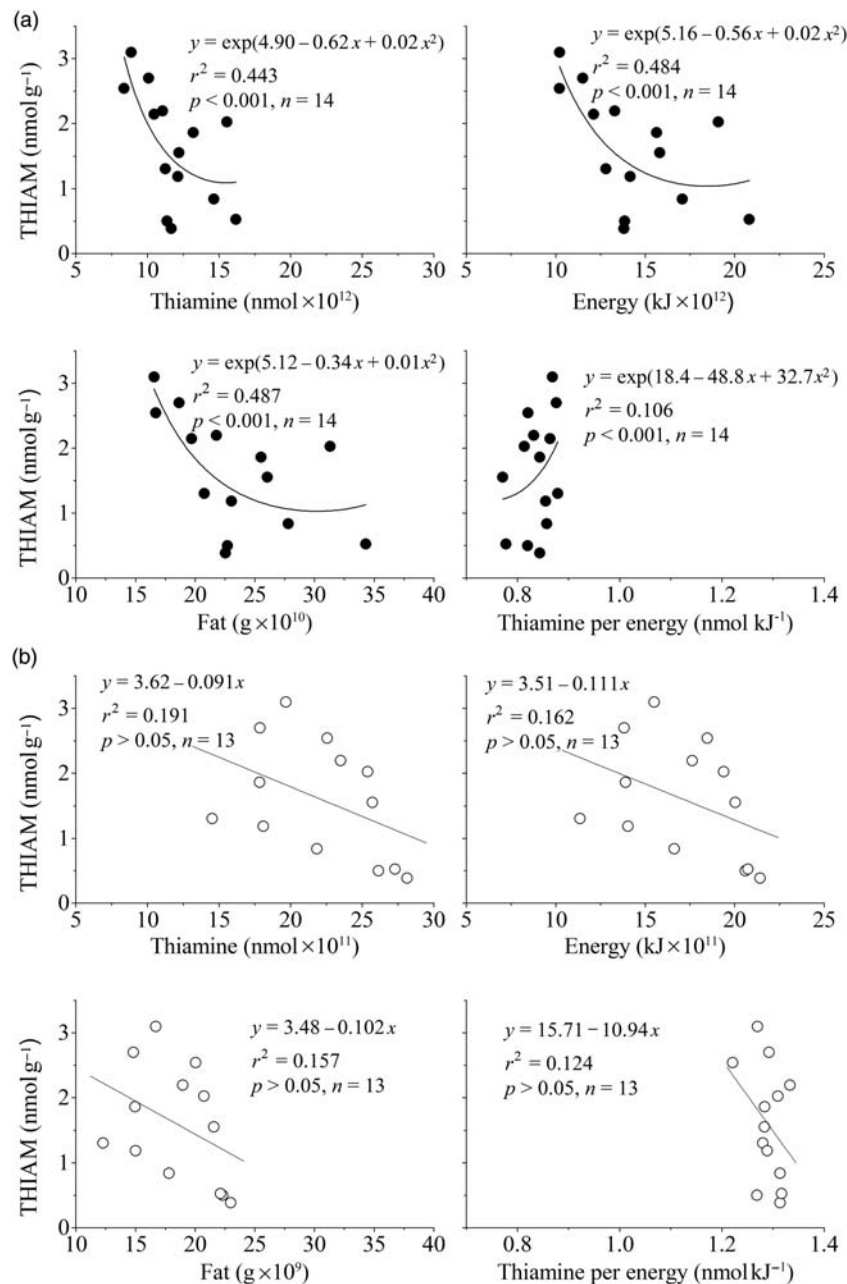
Figure 8. Annual mean free thiamine concentration (THIAM) in unfertilized eggs of M74-monitored salmon from GoB rivers in relation to total thiamine, energy, and fat content, and the thiamine content per unit energy in the total biomass (a) of sprat in the BPr (1993–2006) and (b) of herring (<19 cm) in the BS (1993–2005), with the results of second-order polynomial or linear regression analysis (r^2 = coefficient of determination; n = number of years). The biomasses of sprat and herring (<19 cm) are taken from Mikkonen *et al.* (2011) and derived using datasets from ICES (ICES, 2006a, c).

intake per unit energy also surpassed the suggested limit of requirement for salmon growth (Woodward, 1994) in the southern BPr. During the period of large sprat biomass in the Baltic Sea (ICES, 2006a), the supply of thiamine was high also in the BPr. However, the negative relationship between the THIAM value in salmon eggs and the thiamine content in the Baltic Sea sprat biomass reveals that although salmon prey provided more thiamine during the high incidence of M74, thiamine was depleted from salmon more than in years when there was less M74 present.

Emergence of thiamine deficiency in salmon

Because thiamine is water soluble, it is not preserved in the body for long. In steelhead trout (*O. mykiss*), 11% of the original thiamine dose was still in the muscle after 6 months (Ketola *et al.*, 2008). Therefore, for there to be sufficient thiamine reserves until spawning, the final feeding period is of greatest concern in salmon. By feeding extensively on young sprat in the BPr, salmon obtain sufficient thiamine for growth, but those ascending GoB rivers to spawn have to cope for several months (Ikonen, 2006) with thiamine and antioxidant stores acquired during the

feeding migration. Evidently, because of prespawning fasting, the thiamine concentration in the liver and gonads of salmon decreases during the spawning migration from the southern Baltic to the rivers of the northern GoB (Karlsson *et al.*, 1999b). In contrast, salmon in Gulf of Riga rivers with no M74 spend at least a month less on their spawning migration, ascend spawning rivers later, and supplement their thiamine reserves by feeding on fish species with a lesser fat content than sprat (Karlsson *et al.*, 1999b).

The salmonid thiamine deficiency problems M74 and EMS are clearly associated with large body weight and high CF (Karlsson *et al.*, 1999a; Wolgamood *et al.*, 2005; Werner *et al.*, 2006; Ketola *et al.*, 2009; Mikkonen *et al.*, 2011), which indicate high fat intake (Alvarez *et al.*, 1998; Hemre and Sandnes, 1999). Feeding on a high-fat fish-based diet increases the proportions of polyunsaturated omega-3 fatty acids in general, and the proportion of docosahexaenoic acid (DHA, C22:6n-3; Alvarez *et al.*, 1998; Hemre and Sandnes, 1999; Kjær *et al.*, 2008). This was seen in the eggs of M74 salmon from the Baltic Sea compared with the eggs of salmon feeding in the Atlantic and with no M74 (Pickova *et al.*, 2003). Likewise, foraging of lake trout on alewife containing high levels of DHA was suspected to give rise to the fatty acid composition found in eggs and the incidence of EMS (Czesny *et al.*, 2009). The more diverse diet of salmon in the Northeast Atlantic, consisting, for instance, of crustaceans (Jacobsen and Hansen, 2001) with low levels of fat and unsaturated fatty acids (Hyvönen and Koivistoinen, 1994), is reflected in their proportions of fatty acids (Pickova *et al.*, 2003).

Intra- and interannual variation in the incidence and extent of M74 seems to be explained by differences in the feeding area as well as in annual variation in sprat biomass and recruitment, and hence by feeding on prey with differing quantities of thiamine and lipid. Feeding in various areas of the BPr, and individual differences in feeding behaviour (P. J. Vuorinen, unpublished), can even explain intra-annual variation in the incidence of M74, mainly through differences in the dominance of sprat in the diet. However, salmon can also differ considerably in terms of their retention of fat, despite consuming the same prey (Sargent *et al.*, 2002). By feeding extensively on young sprat, Baltic salmon obtain large quantities of fat and energy, but a very small amount of thiamine per unit energy obtained, and also the lowest quantity of carotenoids. Nevertheless, the more fatty a salmon becomes, the more obvious is the disproportion between its energy and thiamine content, along with carotenoid reserves (seen as a pale colour of flesh and eggs), because polyunsaturated fatty acids are easily oxidized and predispose fish to oxidative damage (Kjær *et al.*, 2008).

Double bonds, of which DHA contains the largest number among fatty acids, react readily with free radicals elicited in normal cell processes (Thomas, 1995). This leads to a chain reaction in which several new different radicals are formed repeatedly (Porter *et al.*, 1995). Carotenoids, as efficient singlet oxygen quenchers, participate in preventing lipid peroxidation (Lundström *et al.*, 1999b), which might also have been a reason for their decrease during the development of M74 yolk-sac fry (Pettersson and Lignell, 1999). Thiamine, interacting directly with free radicals and hydroperoxides (Lukienko *et al.*, 2000) also inhibits lipid peroxidation and free radical oxidation along with other antioxidants, functioning as a site-specific antioxidant (Gibson and Zhang, 2002). Therefore, generalized oxidative stress may reduce the levels of thiamine components and the enzymes that act on thiamine. On the other hand, thiamine deficiency in itself increases oxidative stress (Gibson and Zhang, 2002).

TPP is an essential cofactor for the key enzymes of energy metabolism: transketolase, pyruvate dehydrogenase, and α-ketoglutarate dehydrogenase. Therefore, it has a central role in producing energy in the form of ATP and sustaining the reducing power of cells, i.e. the NADH/NADPH status, and also has a direct linkage to fatty acid metabolism. Each of the TPP-dependent enzyme complexes consists of subunits with the presence of sulph-hydryl groups, which are particularly sensitive to a variety of oxidative stressors (Gibson and Zhang, 2002). In various radical and oxidation reactions, the sulph-hydryl group of thiamine is oxidized and thiamine is irreversibly transformed into thiochrome or thiamine disulphide, which no longer have coenzyme properties (Lukienko *et al.*, 2000; Gibson and Zhang, 2002). This depletes thiamine stores.

To conclude, a deficiency of thiamine in the eggs of salmon, a species which undergoes a long prespawning fasting period, primarily results from an abundant but unbalanced fish diet with too low concentration of thiamine in relation to fat and energy content. The intake of thiamine for Baltic salmon in relation to energy and fat remains lowest by eating young clupeids, most notably young sprat. The thiamine content per unit fat and energy in the diet of salmon has been least during years and in areas where recruitment and biomass of sprat have been high. A low thiamine intake, and lipid peroxidation resulting from an increased intake of unsaturated fatty acids, both cause oxidative stress. Because of lipid peroxidation and the antioxidant property of thiamine, thiamine reserves are further depleted at an increasing rate during the prespawning fasting period, and diminished body stores do not allow adequate deposition of thiamine into developing oocytes during exogenous vitellogenesis. To inhibit M74, great variation in the size of prey stocks utilized by salmon should be avoided. In the BPr, where sprat reproduce, the size of the sprat stock needs to be under control, and possibly not allowed to exceed that of herring. The safest strategy for attaining this objective would be to ensure a large, stable cod stock (Casini *et al.*, 2009), to prey on the sprat. Alternatively, increased fishing on sprat would have the same inhibiting effect on M74.

Acknowledgements

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