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**Author(s):** Pekka J. Vuorinen, Mervi Rokka, Tiina Ritvanen, Reijo Käkelä, Soili Nikonen, Tapani Pakarinen & Marja Keinänen

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**Year:** 2020

**Version:** Published version

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**Please cite the original version:**

Vuorinen, P.J., Rokka, M., Ritvanen, T. et al. Changes in thiamine concentrations, fatty acid composition, and some other lipid-related biochemical indices in Baltic Sea Atlantic salmon (*Salmo salar*) during the spawning run and pre-spawning fasting. *Helgol Mar Res* 74, 10 (2020).  
<https://doi.org/10.1186/s10152-020-00542-9>.


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ORIGINAL ARTICLE

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# Changes in thiamine concentrations, fatty acid composition, and some other lipid-related biochemical indices in Baltic Sea Atlantic salmon (*Salmo salar*) during the spawning run and pre-spawning fasting

Pekka J. Vuorinen<sup>1,2\*</sup> , Mervi Rokka<sup>3</sup>, Tiina Ritvanen<sup>3</sup>, Reijo Käkelä<sup>4</sup>, Soili Nikonen<sup>3</sup>, Tapani Pakarinen<sup>1</sup> and Marja Keinänen<sup>1</sup>

## Abstract

Salmonines in the Baltic Sea and North American lakes suffer from thiamine (vitamin B1) deficiency, which is connected to an abundant lipid-rich diet containing substantial amounts of polyunsaturated fatty acids (PUFAs). In the Baltic region, this is known as the M74 syndrome. It affects both adult salmon (*Salmo salar*) and especially their offspring, impairing recruitment. However, very little is known about the thiamine and lipid metabolism of salmon during feeding and spawning migrations in the Baltic Sea. In this study, salmon females were sampled along the spawning run from the southern Baltic Proper in four locations at sea and finally at spawning in a river at the Bothnian Bay in a year with insignificant M74 mortality. Changes in concentrations of thiamine and its components in muscle, ovaries, and the liver and other biochemical indices potentially relating to lipid and fatty acid metabolism were investigated. The results provide further evidence of the role of peroxidation of PUFAs in eliciting thiamine deficiency in salmon: During the entire spawning run, the muscle total lipid content decreased by 50%, palmitic acid (16:0) by 62%, and docosahexaenoic acid (DHA, 22:6n-3) by 45%. The concentration of total thiamine decreased significantly until the spawning in the liver and ovaries, 66 and 70% respectively. In the muscle, the proportion of thiamine pyrophosphate of total thiamine increased with the use of muscular lipid stores. There was no trend in the concentration of total carotenoids during the spawning run. The doubling of the concentration of hepatic malondialdehyde indicated peroxidation of PUFAs, and the mobilisation of body lipids suppressed the activity of glucose-6-phosphate dehydrogenase, as consumed dietary lipids would also have done.

**Keywords:** Atlantic salmon *Salmo salar*, Carotenoids, Fatty acids, G6PDH, Lipid peroxidation, MDA, M74 syndrome, PUFA, Thiamine deficiency

## Background

The salmonines, fatty fish species in the Baltic Sea (Fig. 1) and North American lakes, suffer from lipid-related thiamine (vitamin B1) deficiency, impairing reproduction [1, 2]. Thiamine deficiency is connected to an abundant lipid-rich fish-based diet [1, 3–5]. Such a diet contains *n-3* series polyunsaturated fatty acids (*n-3* PUFAs) in high

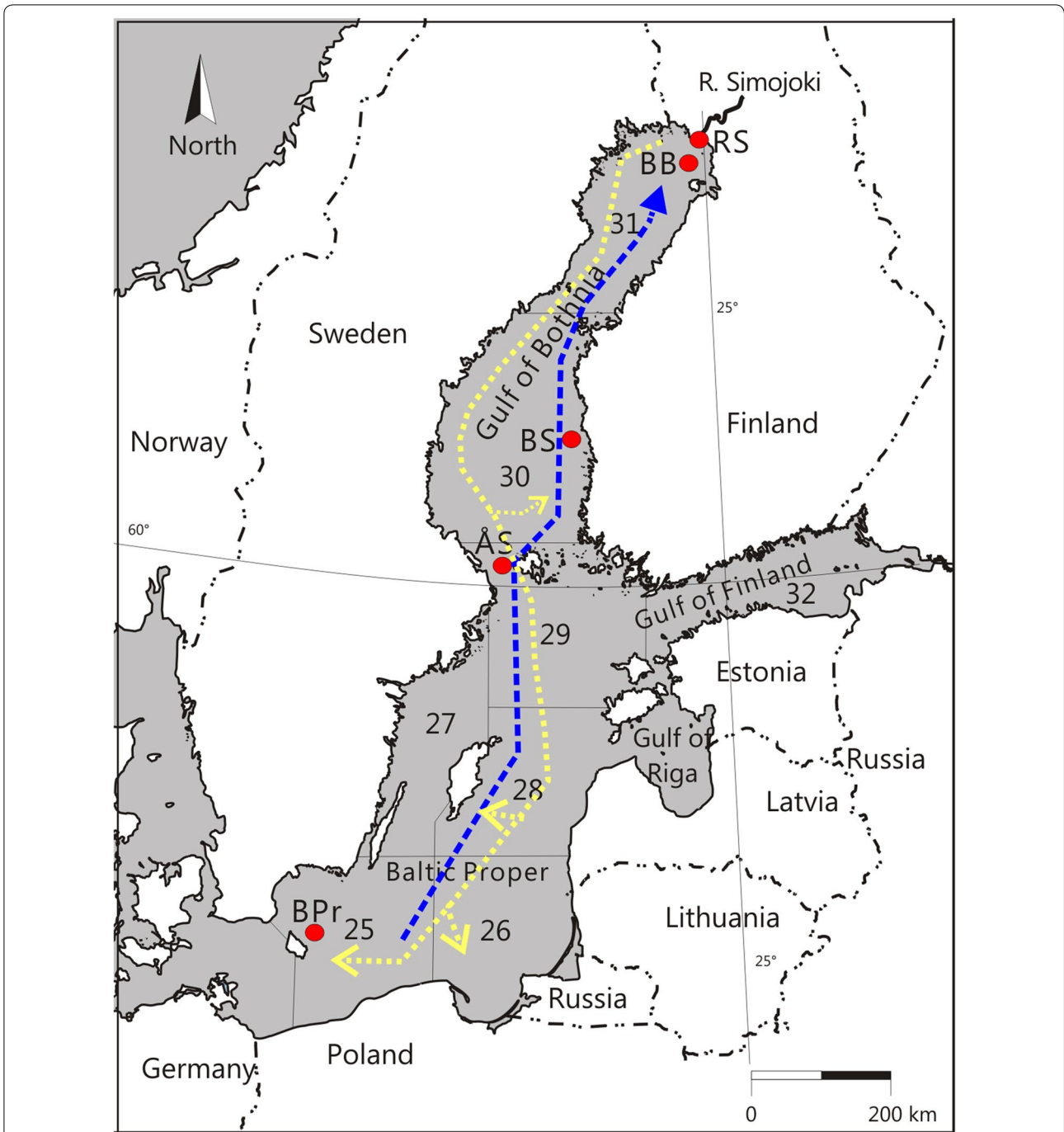
\*Correspondence: ext.pekka.vuorinen@luke.fi; pekka.vuorinen@gmail.com

<sup>1</sup> Natural Resources Institute Finland (Luke), P.O. Box 2, 00791 Helsinki, Finland

Full list of author information is available at the end of the article



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**Fig. 1** The Baltic Sea [with the subdivisions (SD 25–32) of the International Council for the Exploration of the Sea (ICES, origin of the map)]. Two-sea-year-old salmon undergoing the spawning migration were sampled (red dots) from the Baltic Proper (BPr), Åland Sea (ÅS), Bothnian Sea (BS), Bothnian Bay (BB), and spawning salmon females in the River Simojoki (RS). Approximate feeding migration and spawning run are indicated by the yellow and blue broken lines respectively

concentrations [6, 7]. In ascending adult salmonines, thiamine deficiency appears as weakness and uncoordinated swimming, but mortalities have also been found in fasting salmonines before the spawning period [8–11]. Most

clearly, thiamine deficiency manifests in the yolk-sac fry (i.e., eleutheroembryos or free embryos [12]), which must rely on the nutrient reserves of yolk until they start external feeding [13]. If too little thiamine is transported from

the tissues of a female into developing oocytes, a proportion or all of the offspring will die of thiamine deficiency during the yolk-sac phase [2, 4, 11, 14].

In the Baltic Sea region, this thiamine deficiency is known as the M74 syndrome [3, 15]. It has principally affected Atlantic salmon (*Salmo salar* L., hereafter, Baltic salmon or salmon), with its intensity varying annually and to some degree between rivers [3, 4, 11, 16]. According to Finnish M74 monitoring, M74 has had disastrous effects on all salmon stocks around the Gulf of Bothnia (Fig. 1) in the years when the most (up to 92%) of the females have been thiamine-deficient (M74 female) and annually the majority (up to 75%) of the offspring have died [4, 11, 16]. Baltic salmon primarily prey on sprat [*Sprattus sprattus* (L.)] and Baltic herring (*Clupea harengus membras* L., hereafter, herring) during the feeding migration [13]. These two species, with different proportions depending on the feeding area, together constitute more than 90% of salmon stomach content, both by weight and numbers [17, 18]. The principal dietary origin of M74 in years of its high and moderate incidence has been abundant fatty young sprat in the southern Baltic Sea, the Baltic Proper (Fig. 1) [1, 3, 4]. In the years following the collapse of cod (*Gadus morhua* L.) stocks, M74 has peaked because of the high recruitment of sprat and the increase in sprat stocks [3]. Cod is the principal predator of sprat in the Baltic Proper [19, 20], but as a lean fish species, cod themselves have not suffered from M74 [21].

Anadromous brown trout (*Salmo trutta* m. *trutta* L.) have also suffered from thiamine deficiency M74 but to a much lesser degree than salmon [22]. Compared to pelagically feeding salmon, the dietary fish composition of brown trout is more diverse, and the diet also contains benthic invertebrates [22]. Moreover, brown trout is a less fatty species than salmon and thus not as susceptible to dietary lipid-related thiamine deficiency [5]. An equivalent syndrome in many salmonine species of the Great Lakes in North America is called Thiamine Deficiency Complex (TDC) [2, 5], and in Finger Lakes Atlantic salmon, Cayuga Syndrome [23]. These have been connected to preying on alewife [*Alosa pseudoharengus* (Wilson)], which is a fatty species [5, 24], and to strengthening of alewife stocks [25]. Salmon in the Atlantic Ocean or those feeding in the Gulf of Riga (Fig. 1) in the Baltic Sea, which have a more diverse diet than most Baltic salmon [26, 27], have not suffered from thiamine deficiency [28–30].

Most salmon in the Baltic Sea originate from the stocks of the rivers that drain into the Bothnian Bay (Fig. 1), located in the northernmost Baltic Sea [31]. Salmon from these rivers principally migrate for feeding to the Baltic Proper [32]. In the Baltic Proper, the proportion of sprat

in the total biomass of salmon prey has been high since the 1980s, despite some differences in their proportional biomasses between sub-areas [3, 33]. Some salmon from the Bothnian Bay rivers halt to feed in the Bothnian Sea, where herring is the dominant prey fish of salmon [3, 17, 33]. The proportion of salmon that stop to feed in the Bothnian Sea depends on the strength of the newyear class of young herring to be preyed on in the area [34, 35].

The Baltic salmon feeding period in the sea generally lasts 1–4 years, after which they start migration in April–June back to the rivers where they were born or introduced [13]. However, most salmon returning to the rivers of the Bothnian Bay have been feeding for two full growing seasons in the sea (78% of females in the M74 monitoring data of the Natural Resources Institute Finland (Luke) for the years 1985–2018, N=1 454). Salmon on their spawning run reduce their preying and finally stop feeding approximately four months before spawning [18]. During the pre-spawning period, they thus rely on the body's energy reserves. Fatty acids (FAs) hydrolysed from visceral and tissue lipid stores are the preferred source of metabolic energy [36, 37]. Muscular lipid content in salmonine species can decrease before spawning by 40–60%, and the visceral lipids by up to 70% [6]. The decrease in the total body mass from the time of entering the mouth of the rivers of the northern Gulf of Bothnia to the actual spawning was approximately 10% in 1998 and between 2001 and 2006 [3].

Animals must obtain thiamine from their diet [38]. The entire bodies of sprat and herring contain on average several times more thiamine [1, 39] than is required for the proper growth of salmon [40]. However, thiamine deficiency can in general result from a high-calorie diet [38, 41]. The thiamine requirement therefore increases with the increase in the dietary energy of salmonines [40], which, in a fish-based fatty diet, is largely determined by the lipid content [1]. The lipid content, and thus energy density [42], is in general greater in sprat than in herring and is highest in the youngest sprat [1, 7, 39]. An abundant intake of fish lipids increases body lipid content and therefore the concentration of *n*-3 PUFAs in the tissues of fatty fish species [6, 7, 43, 44]. PUFAs, and especially highly unsaturated FAs (HUFAs), are highly susceptible to lipid peroxidation [45, 46]. It has been shown that thiamine reacts with free radicals and hydroperoxides, and is destroyed in these reactions [47]. The youngest and fattiest sprat specimens contain the least thiamine, which is potentially due to their high PUFA content [7, 39]. The supply of thiamine in relation to PUFAs is therefore lowest in the youngest specimens [7].

Thiamine pyrophosphate (TPP) functions as a co-enzyme for at least twenty enzymes, including enzymes

essential for energy production in the tricarboxylic acid cycle [48]. TPP is involved in a step of the pentose phosphate cycle and  $\alpha$ -oxidation of FAs [49, 50]. It also plays an important role in sustaining cellular reducing power by regulating NADH/NADPH balance [51]. Moreover, thiamine serves as a site-specific antioxidant [47, 51], specifically in preventing lipid peroxidation [52], and thiamine is thus depleted in peroxidation reactions [47, 51]. Thiamine monophosphate (TMP) is an essential intermediate in thiamine absorption and transfer between extra- and intracellular compartments [38, 53].

The incidence of M74 [4] is predicted and monitored annually by analysing thiamine in the ovulated unfertilised eggs of salmon (e.g. from the River Simojoki; Fig. 1) [11, 54]. Free thiamine (THIAM) and two phosphorylated thiamine derivatives, TPP and TMP, have been analysed and totalled as total thiamine (TotTHIA) [39, 55]. Although yolk-sac fry mortality also correlates with the concentration of TotTHIA in eggs, the strongest relationship has been recorded with the concentration of THIAM [2, 4, 54]. When it remains  $< 1.0 \text{ nmol g}^{-1}$ , yolk-sac fry mortalities result [11].

The eggs of M74 and TDC salmonines in general also contain decreased concentrations of total carotenoids and astaxanthin [11, 29, 56, 57], which is the most common carotenoid in salmon [58]. Both have been used to indicate general oxidative stress, because carotenoids play an important role in quenching free radicals [59]. However, it is a low concentration of thiamine in eggs that results in M74 symptoms and death in yolk-sac fry after hatching [14, 54, 60, 61], not the depletion of carotenoids [62].

The specific indication of an increased rate of lipid peroxidation is an increase in the concentration of malondialdehyde (MDA), which is the principal peroxidation product of PUFAs [63, 64]. An increase in dietary lipids in general suppresses the activity of glucose-6-phosphate dehydrogenase (G6PDH), which is a key regulatory enzyme in the lipogenic pathway [65]. It is not known how fasting and the mobilisation of lipid reserves of salmon affect G6PDH activity. Due to the mobilisation of lipids for metabolism and oocyte development, lipophilic organohalogen translocate, and their concentrations in salmon muscle therefore increase during pre-spawning fasting [18]. Baltic salmon still contain high concentrations of organohalogenes such as PCBs [18, 66, 67], and their concentrations were also measured in the salmon of the present study [18]. For example, in rats, PCBs in large doses have been demonstrated to decrease thiamine concentrations and elicit changes in fatty acid composition [68, 69]. Some organohalogenes induce 7-ethoxyresorufin-O-deethylase (EROD) activity, and this has been used as a biomarker for them [70, 71].

The aim of this research was to reveal how the concentration of thiamine and its components in different tissues, as well as other lipid-related biochemical indices of salmon, changed during the spawning run from the southern Baltic Sea to the spawning rivers of the Bothnian Bay. Salmon were sampled in four sea locations during May–June, and finally, during the autumn spawning in October in salmon that had returned to the River Simojoki (Fig. 1), which they had reached between early June early July. The background knowledge is that thiamine deficiency in salmon is linked to dietary and body lipids, and PUFAs [1, 2, 7] and lipid peroxidation therefore cause oxidative stress [44]. The total lipid content, FAs, and total carotenoids in muscle, as well as the concentrations of MDA and activities of G6PDH in the liver, were investigated in the same salmon at these locations. Moreover, the EROD activity in the liver was measured, because the concentration of the organochlorines of these salmon was known [18]. Our hypotheses were that the decrease in the thiamine concentrations would already be detected during the spawning migration, and that the muscle FA composition would change, because metabolic energy was provided through  $\beta$ -oxidation of FAs of body lipids during pre-spawning fasting. Changes in salmon lipid content and FA composition were expected to be linked with other measured biochemical indices, allowing verification of the understanding of the correlational relationships between dietary lipids and PUFAs, and the incidence of thiamine deficiency M74 in salmon.

## Materials and methods

### Salmon and sampling

Samples of Atlantic salmon females on their spawning migration in the Baltic Sea were collected in 2004, as described by Vuorinen et al. [18] when reporting organohalogen concentrations of these same salmon specimens. Briefly, the first samples were taken from salmon caught from the southern Baltic Proper near Bornholm on 16 May, and then along the Finnish coast to the mouth of the spawning rivers in the northern Gulf of Bothnia in May–June (Fig. 1). The names of the sampling locations and the sampling dates are given in Table 1. Professional fishermen caught the fish, and the staff of the Luke (previously the Finnish Game and Fisheries Research Institute, FGRI) performed the sampling in cooperation with the fishermen. Only those salmon females that had been feeding two years in the sea (2 sea-year salmon) and those sampling locations where the number of salmon sampled for FA analysis was at least four per site (i.e.,  $N = 4$ –10) were accepted for this study ( $N = 37$  in total). The final samples were taken during the spawning period in October from female fish that had been caught by trap



**Table 1** The mean ( $\pm$  SE) total mass, total length, and muscle lipid content of salmon

Location	Distance km	Sampling date	Body mass kg		Body length cm		Muscle lipid %	
			Mean $\pm$ SE	N	Mean $\pm$ SE	N	Mean $\pm$ SE	N
Baltic Proper (BPr)	0	19 May 2004	6.7 $\pm$ 0.5 <sup>a</sup>	10	86.7 $\pm$ 1.4 <sup>a</sup>	10	7.29 $\pm$ 0.88 <sup>b</sup>	10
Åland Sea (ÅS)	585	2 June 2004	5.6 $\pm$ 0.2 <sup>a</sup>	10	87.4 $\pm$ 0.9 <sup>a</sup>	10	9.48 $\pm$ 0.64 <sup>b</sup>	10
Bothnian Sea (BS)	765	3 June 2004	5.8 $\pm$ 0.9 <sup>a</sup>	4	84.3 $\pm$ 4.6 <sup>a</sup>	4	7.57 $\pm$ 0.88 <sup>b</sup>	4
Bothnian Bay (BB)	1250	11 June 2004	5.8 $\pm$ 0.3 <sup>a</sup>	7	84.3 $\pm$ 1.5 <sup>a</sup>	7	8.12 $\pm$ 1.05 <sup>b</sup>	7
River Simojoki (RS)	1400	6 October 2004	5.5 $\pm$ 0.5 <sup>a</sup>	5	87.7 $\pm$ 1.6 <sup>a</sup>	6	3.65 $\pm$ 0.78 <sup>a</sup>	6

The 2-sea-year-old Baltic salmon were sampled during the spawning run from the southern Baltic Sea to the rivers running into the Bothnian Bay. Significant differences ( $p < 0.05$ , post hoc SNK in one-way ANOVA) in the means between the sampling locations are indicated with no common letter as a superscript to the SE value of the appropriate mean. The number of specimens (N) is also given

net while ascending the River Simojoki in the north-eastern corner of the Bothnian Bay. These fish were held in basins with through-flowing river water at the Kemnmaa hatchery of Luke until the stripping of ovulated eggs during the spawning period. In the Finnish M74 monitoring, mortalities of offspring of these salmon were daily observed and registered from hatching until the end of the yolk-sac phase in incubation at the laboratory of FGFR1 in Helsinki [11, 15, 16]. If the thiamine concentration of eggs was small and typical symptoms in yolk-sac fry were detected, the female was classified as a M74 female.

The total length of the salmon was measured at sampling and they were weighed, but the mass was not obtained in one case (one among six from the River Simojoki salmon) (Table 1). Scale samples were taken for age determination. For biochemical analyses, a 10 g piece of distal liver (while carefully avoiding piercing the gallbladder), an approximately 10 cm wide cross-sectional sample of the fish cut around the dorsal fin, and a 20 g section of the mid-ovaries of salmon caught from the sea were sealed separately in coded polyethylene bags and immediately frozen in dry ice. Eggs from mature, spawning females were stripped on a sieve to filter off the ovarian fluid, and approximately 100 ml of unfertilized eggs were closed in a coded zip-lock plastic bag and frozen. All the samples were sent frozen with dry ice to the laboratory, where they were stored at  $-80$  °C until analysis (for 4–6 weeks). In the laboratory, the samples for analyses of thiamine, FAs and carotenoids were cut in the frozen state from the left epaxial muscle of a slice of salmon. Muscle lipid content values were obtained from the lipid extraction procedure for the analysis of concentrations of organohalogenes in these salmon [18].

The feeding area of all the sampled salmon was determined on the basis of the ratio of the total concentration of polychlorinated dibenzo-*p*-dioxins plus dibenzofurans (PCDD/F) to the concentration of coplanar polychlorinated biphenyls (CoPCB) [18, 66] and that of spawning

salmon in the River Simojoki also using FA signatures [4]. According to the FA signatures and the ratio of organochlorines, all the salmon except one sampled in the Bothnian Bay and one, the M74 female, in the River Simojoki had been feeding in the Baltic Proper. In comparing the salmon from the Baltic Proper and River Simojoki, the M74 female was excluded from the calculations.

#### Fatty acid analysis

For the FA analysis, the lipids of the salmon muscle were extracted using the Schmid-Bondzynski-Ratzlaff method (ISO 1735/IDF 5: 2004). The FAs were analyzed, as in Keinänen et al. [7], in the laboratory of the Finnish Food Authority. The extracted total lipids were transesterified in methanolic boron trifluoride, according to Slover and Lanza [72]. The same lot of boron trifluoride was used in every run. The formed FA methyl esters (FAMES) were analysed by gas-liquid chromatography (Agilent HP 5890) employing an HP Innowax capillary column (30 m, I.D. 0.32 mm, film thickness 0.5  $\mu$ m) and flame-ionization detection. Individual FAMES were identified on the basis of their relative retention times against a FAME standard mixture.

The FA results are presented as proportions (percentage values or relative amounts) based on their FAME peak area as a proportion of the total area of all the integrated chromatographic peaks. The concentrations of FAs were calculated from the proportions of each FA and the lipid-%. The FAs were classified into the structural categories of saturated FAs (SFAs), monounsaturated FAs (MUFAs), and PUFAs, and the latter were further divided into the sums of *n*-3 PUFAs and *n*-6 PUFAs, similarly as in Keinänen et al. [7]. As *n*-3 HUFAs, we classified docosahexaenic acid (DHA, 22:6*n*-3), eicosapentaenoic acid (EPA, 20:5*n*-3), and docosapentaenoic acid (DPA, 22:5*n*-3), which fulfilled the HUFA criteria possessing 20 carbon atoms and three double bonds at a minimum [36]. HUFAs include, in addition, arachidonic acid (ARA, 20:4*n*-6). Of the identified 21 FAs (Additional

file 1) for the principal component analysis (PCA) those 16 FAs were included, for which the mean proportions were >0.5% (area%, which is close to wt%).

#### Thiamine analysis

Thiamine components were analyzed in the muscle, liver, and ovaries (or eggs of mature fish) of salmon by high-performance liquid chromatography (HPLC), according to Vuorinen et al. [39] with Empower1 Chromatography Manager. Thiamine components comprised the phosphorylated thiamine derivatives, TPP and TMP, and unphosphorylated thiamine, THIAM, which were summed up as TotTHIA. Proportions of TPP, TMP, and THIAM of TotTHIA were also calculated.

A subsample of the laboratory control sample (salmon egg homogenate) was processed and analysed along with the samples for quality assurance. No certified reference material for thiamine is available.

#### Analysis of carotenoids

The total carotenoid concentration was determined in the salmon muscle. The concentrations were measured spectrophotometrically, according to Pettersson and Lignell [58], with slight modifications. A sample of circa 0.5 g was weighed and homogenized (IKA Eurostar) with 2 ml of acetone. The homogenization tube was rinsed with 1 ml acetone, which was combined with homogenate. Cyclohexane (3 ml) and distilled water (1.5 ml) were added and mixed thoroughly. The mixture was centrifuged at  $1500 \times g$  for 15 min at 10 °C (Beckman Avanti J-30 I). The cyclohexane phase was separated and absorbance measured at 474 nm (Shimadzu UV-2401 PC). Calculation of the carotenoid concentration, according to the Lambert–Beer law, was based on an extinction coefficient of  $195 \text{ cm}^{-2} \text{ mg}^{-1}$  [58].

#### Malondialdehyde analysis and enzyme activity determinations

Hepatic microsomes were obtained, according to Beyer et al. [73], with slight modifications. Approximately 0.5 g of liver was weighed and rinsed for 10 s in the homogenization buffer (150 mM KCl in 50 mM Tris, pH 7.4), a volume of four times of buffer in relation to the sample weight was added (1 + 4), and the liver was homogenised in a Potter-Elvehjem glass-Teflon homogeniser. Homogenate was centrifuged (Beckman Avanti-J 30 I) at  $14\,000 \times g$  for 20 min at 4 °C and the supernatant was further centrifuged (Beckman Avanti-J 30 I) at  $105\,000 \times g$  for 60 min at 4 °C to separate the microsome fraction for the EROD activity determination. The supernatant was used in G6PDH (EC 1.1.1.49) activity determination and to measure the peroxidation potential as the MDA concentration.

For the measurement of the MDA concentration, the following reagents were prepared: 10.3 mM 1-methyl-2-phenyl-indole in acetonitrile, diluted 1:3 with methanol (R1), 37% HCl (R2), and for the standard curve, 10 mM 1,1,3,3-tetramethoxypropane in 20 mM Tris–HCl buffer (S2). For the determination, the following amounts were pipetted into an Eppendorf vial: 650  $\mu\text{l}$  R1, 100  $\mu\text{l}$  supernatant (sample, see above), 100  $\mu\text{l}$  distilled water and 150  $\mu\text{l}$  R2. The vial was vortexed, incubated in a water bath at 45 °C for 40 min, cooled in ice, centrifuged at  $15\,000 \times g$  for 10 min, and absorbance in the supernatant was measured at 586 nm (Shimadzu P2450). The result was calculated from the absorbance against the four-point standard curve, with the standard solutions (S2) treated similarly to the actual samples.

The activity of G6PDH was determined according to the instructions of a commercial kit (Sigma-Aldrich, Trinity Biotech No. 345).

The EROD activity was measured in liver microsomes. The analysis was performed using a modification of the method described by Stagg and McIntosh [74]. EROD activity was measured with a 96-well microplate reader (Victor<sup>3</sup> Multilabel Plate Reader 1420, Perkin Elmer, excitation: 535 nm; emission: 585 nm) after a 5-min incubation at 30 °C in 1 ml of a reaction mixture containing ethoxyresorufin as substrate. NADPH was required to initiate the reaction, and it was stopped by the addition of 2 ml of cold acetone [75]. The EROD activity was normalised to protein content in the microsomes and expressed as pmol produced resorufin  $\text{min}^{-1} \text{ mg protein}^{-1}$ . The protein content was measured with a 96-well microplate reader (GENios, Tecan) and compared with a bovine serum albumin standard. The protein analysis method was a modification of the method described by Bradford [76].

The quality of biochemical analyses was ascertained by inspecting relative standard deviations of parallel samples and reanalysing samples if needed.

#### Statistical analysis

One-way ANOVA with SNK post hoc test ( $p < 0.05$ ) was applied to test the differences between the salmon groups sampled through the spawning route in the biochemical proxies and in the total length and mass of salmon. When comparing the FA differences between the locations, one-way ANOVA with Bonferroni adjustment for the group comparisons was applied. A general linear model was applied to test the muscle lipid content and the concentration of TotTHIA in the liver, ovaries, and muscle, and concentration of muscular carotenoids, against the migration distance. It was also applied to investigate relationship between TotTHIA in the ovaries and the liver or muscle and between total carotenoids and TotTHIA

in ovaries, liver, or muscle. In all these calculations, untransformed percentage values were used [77].

The PCA [78] was used for the multivariate statistical comparisons of the FA profiles of salmon caught along their spawning run from the Baltic Proper to the spawning river. Prior to the PCA, the FA data were arc-sine transformed and subsequently standardized (variable deviations homogenized) to prevent the abundant components with large variance from dominating the analysis. A biplot graph based on PCA was constructed to illustrate the compositional similarities/differences among and between the groups and to indicate correlations between the variables. Soft independent modeling of class analogy (SIMCA) was used to quantify the compositional differences between the fish groups at the  $p < 0.05$  level [79].

The PCA and SIMCA were performed with Sirius software (ver. 8.5, Pattern Recognition Systems, Norway), and the other statistical analyses with Statistical Analysis System (ver. 9.4) software (SAS Institute Inc.).

## Results

### Salmon characteristics

The mean body mass and length of salmon on the spawning migration did not differ between the sampling locations (Table 1). The muscle lipid content was significantly smaller in salmon that ascended the river and were sampled during the spawning period than in all the other salmon groups (Table 1). At sea, the lipid content was 2.0–2.6 times larger than during the spawning period in the river. Calculated from the linear model: [muscle lipid] = 8.690 – 0.002 \* [distance] ( $F_{3,1} = 0.86$ ,  $p > 0.05$ ,  $R^2 = 0.224$ ) muscle lipid content decreased by 35% during the sea phase of the spawning run. However, the total decrease in the lipid content was higher, 50%, when comparing salmon from the first sampling place, the Baltic Proper in May, to salmon at spawning in October.

### Fatty acid composition in muscle

Oleic acid (18:1*n*-9) was the most abundant FA in salmon muscle, DHA was the second, and palmitic acid (16:0) the third most abundant FA in both migrating and spawning salmon. However, their proportions (%; i.e. relative amounts) changed during the pre-spawning fasting as these FAs were used at different rates (Additional file 2).

The proportion of 16:0, especially, but also that of a minor SFA myristic acid (14:0), decreased strongly by the spawning time, while the proportion of stearic acid (18:0), the second common SFA after 16:0, did not demonstrate any clear trend (Fig. 2). The proportions of 16:0 and 14:0 were statistically significantly smaller at the spawning time than in salmon caught at sea. However, their proportions were already significantly smaller in

the salmon from the last sampling location in the sea, the Bothnian Bay, than earlier during the sea phase of the spawning run (Additional file 2a). The relative amount of 18:1*n*-9 did not differ between the sampling locations.

The proportions of all individual HUFAs increased by the spawning time (Fig. 2). Thus, the proportions of DHA, and likewise those of EPA, DPA, and ARA, were statistically significantly larger or tended to be larger in the spawning salmon than in the salmon at any location along the spawning migration route. Of the minor *n*-3 PUFAs, stearidonic acid (18:4*n*-3) was less common in spawning salmon than in the other locations (Additional file 2a).

When addressing the structural categories of FAs, in spawning salmon the proportion of PUFAs was significantly larger ( $p < 0.05$ ) and the proportion of SFAs clearly smaller ( $p < 0.05$ ) than in salmon at the start of the spawning run (Fig. 3 left) or other sampling places in the sea. The proportion of SFAs was also lower in the salmon from the Bothnian Bay than in the other locations ( $p < 0.05$ , not shown). In the other locations in the sea these differences appeared to be less clear. The proportion of *n*-3 PUFAs was also statistically significantly ( $p < 0.05$ ) larger during the spawning period than at the start (Fig. 3 left) or during the sea phase of the spawning migration. The proportions of MUFAs and *n*-6 PUFAs did not differ ( $p > 0.05$ ; Fig. 3 left) between salmon from different places.

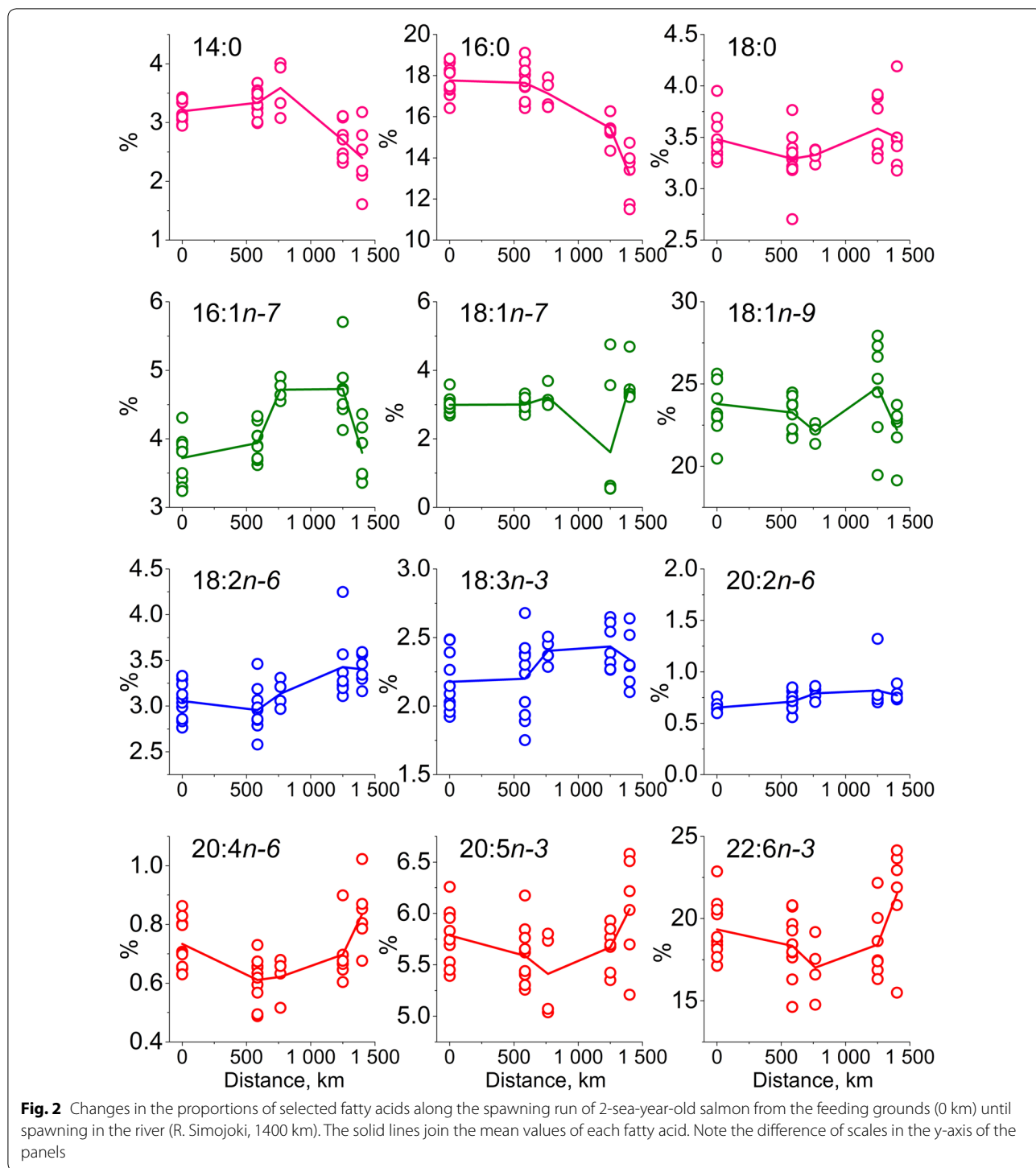
The *n*-3 PUFAs/*n*-6 PUFAs or DHA/EPA ratios did not differ significantly between the salmon groups (not shown).

The concentrations (i.e. the absolute amount per unit of tissue, mg g<sup>-1</sup>) of FAs in the spawning salmon were significantly smaller than in the salmon caught during the sea phase of the spawning run (Additional file 2b). When comparing the spawning salmon from the River Simojoki that had been feeding in the Baltic Proper to the salmon caught from the Baltic Proper at the beginning of the spawning run, the decreases among the structural categories were 60% in SFAs, 49% in MUFAs, and 46% in PUFAs (Fig. 3 right). The concentrations of the individual FAs for these salmon decreased by 32–64%; the decrease was 62% in 16:0, 52% in 18:1*n*-9 and 45% in ARA and DHA (Additional file 2b). The concentrations of the three major FAs, 16:0, 18:1*n*-9, and DHA, in the salmon did not differ statistically ( $p > 0.05$ ) between the sampling places during the sea phase of the spawning run (Additional file 2b).

### Total thiamine concentrations

The concentration of TotTHIA was significantly larger in the liver than in the ovaries and muscle (Table 2, Fig. 4). In ovaries, it tended to be larger than in the muscle

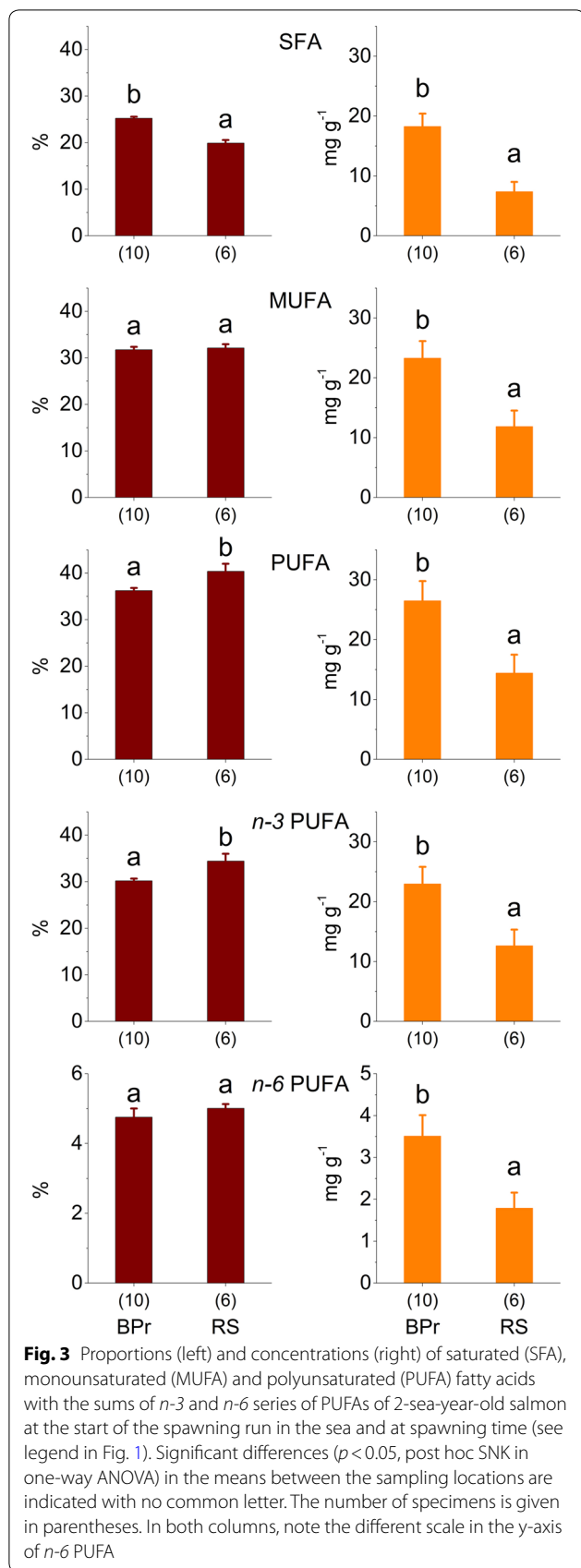




during the sea migration, but at spawning time it was smaller in ovulated eggs than in the muscle (Table 2).

The TotTHIA concentration of the liver was highest in the salmon caught at the start of the spawning run in the southern Baltic Proper, and was significantly lower in salmon during spawning time in the River Simojoki

compared to all other locations (Fig. 4). The decrease in the TotTHIA concentration of the liver was clear according to a linear model (Fig. 5). Thus, the concentration of hepatic TotTHIA decreased on average by 44% until spawning. However, when comparing the Baltic Proper females with those spawning females that had been



feeding in the Baltic Proper the decrease was 66%. The TotTHIA concentration of the ovaries also decreased significantly, by 42%, until spawning according to a linear model (Fig. 5). The concentration of TotTHIA was 70% lower in the eggs of those salmon that had been feeding in the Baltic Proper compared to the ovaries of salmon caught in the Baltic Proper. No significant decrease in the TotTHIA concentration of the muscle was found among the locations (Fig. 5).

There were clear relationships in the TotTHIA concentration between the ovaries and the liver and also between the ovaries and muscle. If the eggs of spawning salmon were included, the concentration of TotTHIA of the ovaries was related to that of the liver according to the linear model: [Ovaries TotTHIA] = - 0.162 + 0.401 \* [Liver TotTHIA],  $F_{1,34} = 37.4$ ,  $p < 0.0001$ ,  $R^2 = 0.501$  and the linear model for the relationship between the ovaries and muscle: [Ovaries TotTHIA] = 0.994 + 0.910 \* [Muscle TotTHIA],  $F_{1,35} = 17.5$ ,  $p < 0.001$ ,  $R^2 = 0.314$ . If the spawning salmon were excluded from the data, the respective models were still statistically significant (Additional file 3).

### Thiamine component concentrations and proportions

In general, the concentration of TPP, except at spawning, was significantly larger in the liver than in the muscle and ovaries (Table 2). The hepatic concentrations of the phosphorylated thiamine derivatives, TPP and TMP, decreased significantly along the spawning run, but the decrease in the THIAM concentration was not so obvious. However, the hepatic THIAM concentration of salmon was larger in the Baltic Proper than in all the other sampling points (Fig. 4).

The concentrations of all thiamine components in the eggs of spawning salmon were lowest compared to the ovaries of salmon along the sea-phase of the spawning run. In the eggs of the single M74 female, the concentration of THIAM was 0.16 nmol g<sup>-1</sup>, and all of its offspring died. In the other, i.e. non-M74 females, the concentration of THIAM ranged 1.44–2.82 nmol g<sup>-1</sup> (N = 5).

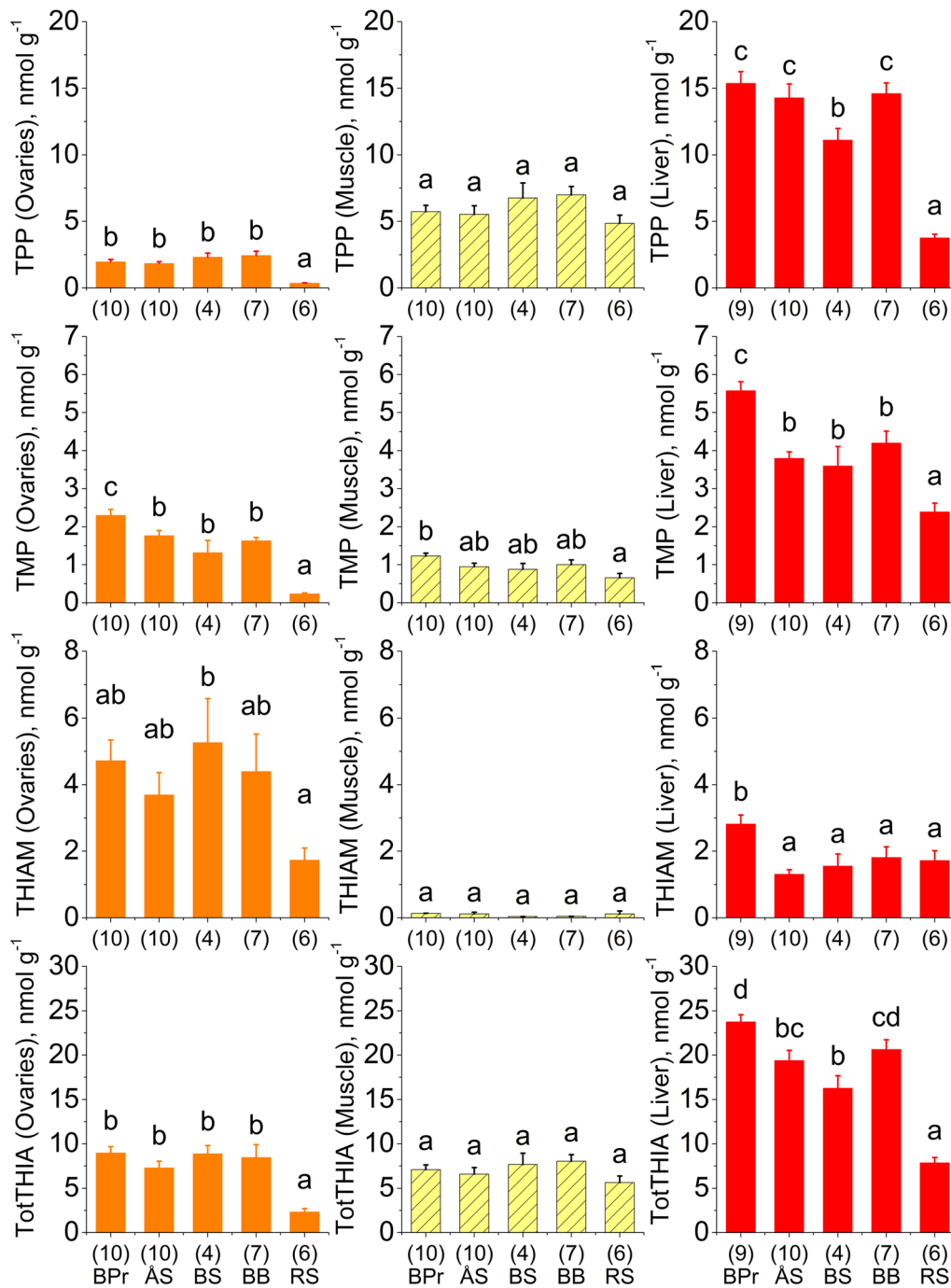
The concentration of THIAM in the muscle was similar and very small in the salmon from all locations (Fig. 4). The muscular concentration of TPP was larger than that of THIAM but did not differ significantly between the sampling locations (Fig. 4).

Nearly all muscular thiamine appeared as TPP (Fig. 6), and the proportion of TPP relative to TotTHIA was always larger in the muscle than in the other tissues (Table 2). The proportion of muscular TPP was larger in the spawning salmon than in the salmon caught in the Baltic Proper. Among the studied tissues, the proportion of THIAM was largest in the ovaries (Table 2) at all sampling locations and tended to be the largest in the eggs at

**Table 2 Differences in concentrations and proportions of thiamine and its components between the liver, muscle, and ovaries**

	Liver		Muscle		Ovaries	
TPP, nmol g <sup>-1</sup>						
Baltic Proper (BPr)	15.364 ± 0.876 <sup>c</sup>	9	5.732 ± 0.466 <sup>b</sup>	10	1.959 ± 0.176 <sup>a</sup>	10
Åland Sea (ÅS)	14.268 ± 1.045 <sup>c</sup>	10	5.521 ± 0.660 <sup>b</sup>	10	1.836 ± 0.133 <sup>a</sup>	10
Bothnian Sea (BS)	11.105 ± 0.868 <sup>c</sup>	4	6.757 ± 1.124 <sup>b</sup>	4	2.306 ± 0.315 <sup>a</sup>	4
Bothnian Bay (BB)	14.604 ± 0.800 <sup>c</sup>	7	6.988 ± 0.636 <sup>b</sup>	7	2.434 ± 0.323 <sup>a</sup>	7
River Simojoki (RS)	3.761 ± 0.273 <sup>b</sup>	6	4.854 ± 0.606 <sup>b</sup>	6	0.347 ± 0.033 <sup>a</sup>	6
TMP, nmol g <sup>-1</sup>						
Baltic Proper (BPr)	5.575 ± 0.236 <sup>c</sup>	9	1.228 ± 0.078 <sup>a</sup>	10	2.294 ± 0.159 <sup>b</sup>	10
Åland Sea (ÅS)	3.796 ± 0.173 <sup>c</sup>	10	0.950 ± 0.091 <sup>a</sup>	10	1.761 ± 0.136 <sup>b</sup>	10
Bothnian Sea (BS)	3.594 ± 0.514 <sup>b</sup>	4	0.879 ± 0.153 <sup>a</sup>	4	1.313 ± 0.324 <sup>a</sup>	4
Bothnian Bay (BB)	4.199 ± 0.313 <sup>c</sup>	7	1.001 ± 0.127 <sup>a</sup>	7	1.628 ± 0.085 <sup>b</sup>	7
River Simojoki (RS)	2.387 ± 0.230 <sup>b</sup>	6	0.657 ± 0.112 <sup>a</sup>	6	0.234 ± 0.023 <sup>a</sup>	6
THIAM, nmol g <sup>-1</sup>						
Baltic Proper (BPr)	2.814 ± 0.271 <sup>b</sup>	9	0.128 ± 0.011 <sup>a</sup>	10	4.719 ± 0.619 <sup>c</sup>	10
Åland Sea (ÅS)	1.303 ± 0.138 <sup>b</sup>	10	0.118 ± 0.048 <sup>a</sup>	10	3.686 ± 0.671 <sup>c</sup>	10
Bothnian Sea (BS)	1.552 ± 0.363 <sup>a</sup>	4	0.034 ± 0.004 <sup>a</sup>	4	5.257 ± 1.328 <sup>b</sup>	4
Bothnian Bay (BB)	1.811 ± 0.319 <sup>a</sup>	7	0.041 ± 0.005 <sup>a</sup>	7	4.392 ± 1.127 <sup>b</sup>	7
River Simojoki (RS)	1.715 ± 0.301 <sup>b</sup>	6	0.119 ± 0.083 <sup>a</sup>	6	1.733 ± 0.363 <sup>b</sup>	6
TotTHIA, nmol g <sup>-1</sup>						
Baltic Proper (BPr)	23.752 ± 0.799 <sup>b</sup>	9	7.087 ± 0.545 <sup>a</sup>	10	8.972 ± 0.703 <sup>a</sup>	10
Åland Sea (ÅS)	19.367 ± 0.153 <sup>b</sup>	10	6.589 ± 0.742 <sup>a</sup>	10	7.284 ± 0.743 <sup>a</sup>	10
Bothnian Sea (BS)	16.251 ± 1.421 <sup>b</sup>	4	7.671 ± 1.270 <sup>a</sup>	4	8.876 ± 0.916 <sup>a</sup>	4
Bothnian Bay (BB)	20.614 ± 1.129 <sup>b</sup>	7	8.029 ± 0.752 <sup>a</sup>	7	8.453 ± 1.448 <sup>a</sup>	7
River Simojoki (RS)	7.863 ± 0.589 <sup>c</sup>	6	5.629 ± 0.739 <sup>b</sup>	6	2.314 ± 0.396 <sup>a</sup>	6
Proportion of TPP, %						
Baltic Proper (BPr)	64.4 ± 1.9 <sup>b</sup>	9	80.5 ± 0.7 <sup>c</sup>	10	22.8 ± 2.2 <sup>a</sup>	10
Åland Sea (ÅS)	73.2 ± 1.5 <sup>b</sup>	10	83.3 ± 1.3 <sup>c</sup>	10	26.7 ± 2.6 <sup>a</sup>	10
Bothnian Sea (BS)	68.7 ± 3.1 <sup>b</sup>	4	88.0 ± 0.6 <sup>c</sup>	4	27.8 ± 6.3 <sup>a</sup>	4
Bothnian Bay (BB)	71.1 ± 2.2 <sup>b</sup>	7	87.2 ± 0.7 <sup>c</sup>	7	30.5 ± 2.4 <sup>a</sup>	7
River Simojoki (RS)	48.5 ± 3.8 <sup>b</sup>	6	86.8 ± 1.7 <sup>c</sup>	6	19.9 ± 6.3 <sup>a</sup>	6
Proportion of TMP, %						
Baltic Proper (BPr)	23.6 ± 0.9 <sup>b</sup>	9	17.6 ± 0.6 <sup>a</sup>	10	26.1 ± 1.5 <sup>b</sup>	10
Åland Sea (ÅS)	20.0 ± 1.0 <sup>b</sup>	10	14.7 ± 0.6 <sup>a</sup>	10	25.9 ± 2.6 <sup>c</sup>	10
Bothnian Sea (BS)	22.0 ± 1.8 <sup>a</sup>	4	11.5 ± 0.6 <sup>a</sup>	4	15.5 ± 4.3 <sup>a</sup>	4
Bothnian Bay (BB)	20.4 ± 1.2 <sup>ab</sup>	7	12.2 ± 0.7 <sup>a</sup>	7	23.8 ± 4.9 <sup>b</sup>	7
River Simojoki (RS)	30.2 ± 1.1 <sup>b</sup>	6	11.4 ± 0.7 <sup>a</sup>	6	11.9 ± 2.1 <sup>a</sup>	6
Proportion of THIAM, %						
Baltic Proper (BPr)	12.1 ± 1.3 <sup>b</sup>	9	1.8 ± 0.1 <sup>a</sup>	10	51.0 ± 3.4 <sup>c</sup>	10
Åland Sea (ÅS)	6.8 ± 0.7 <sup>a</sup>	10	1.9 ± 0.8 <sup>a</sup>	10	47.5 ± 4.3 <sup>b</sup>	10
Bothnian Sea (BS)	9.3 ± 1.5 <sup>a</sup>	4	0.5 ± 0.1 <sup>a</sup>	4	56.6 ± 9.8 <sup>b</sup>	4
Bothnian Bay (BB)	8.6 ± 1.3 <sup>a</sup>	7	0.5 ± 0.0 <sup>a</sup>	7	45.7 ± 6.6 <sup>b</sup>	7
River Simojoki (RS)	21.3 ± 2.8 <sup>b</sup>	6	1.9 ± 1.1 <sup>a</sup>	6	68.2 ± 8.4 <sup>c</sup>	6

Significance of differences ( $p < 0.05$ , post hoc SNK in one-way ANOVA), indicated with no common letter, in the concentrations (means ± SE, nmol g<sup>-1</sup>) of total thiamine, TotTHIA, and its three components (thiamine pyrophosphate, TPP, thiamine monophosphate, TMP and free thiamine, THIAM) and the proportions (means ± SE, %) of thiamine components of the total thiamine between the liver, muscle, and ovaries of 2-sea-year-old Baltic salmon during the spawning run at five locations

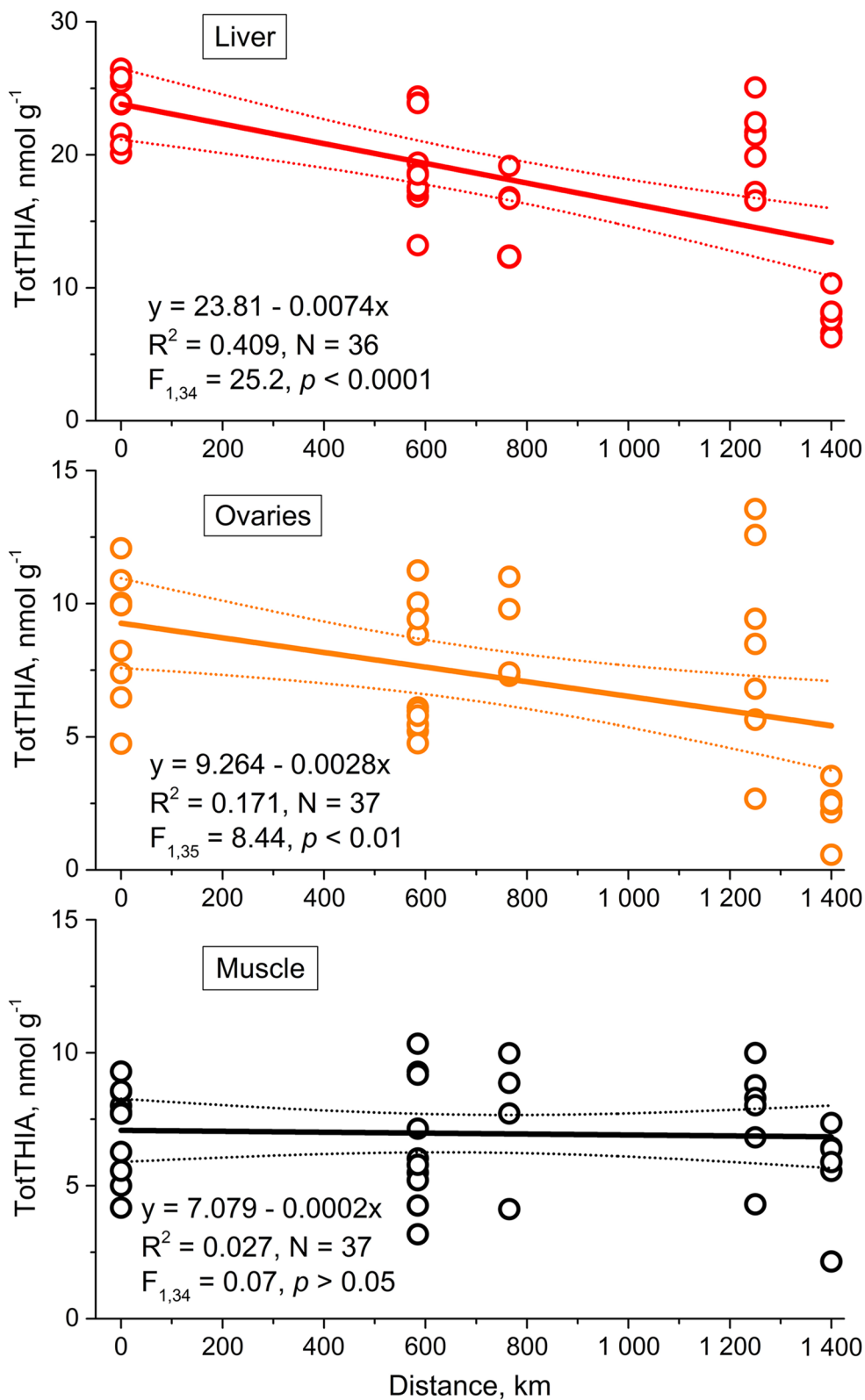


**Fig. 4** Mean (+SE) concentrations of thiamine components (thiamine pyrophosphate, TPP, thiamine monophosphate, TMP and free thiamine, THIAM) and total thiamine (TotTHIA) in ovaries, liver, and muscle of 2-sea-year-old salmon at different sampling locations (see legend in Fig. 1). Significant differences ( $p < 0.05$ , post hoc SNK in one-way ANOVA) in the means between the locations are indicated with no common letter. The number of specimens is given in parentheses. Notice the difference of scales in the y-axis of the panels

spawning (Fig. 6). TPP consistently had the highest proportion in the liver across all locations; however, the proportion of TPP declined while the proportion of THIAM increased by spawning (Fig. 6).

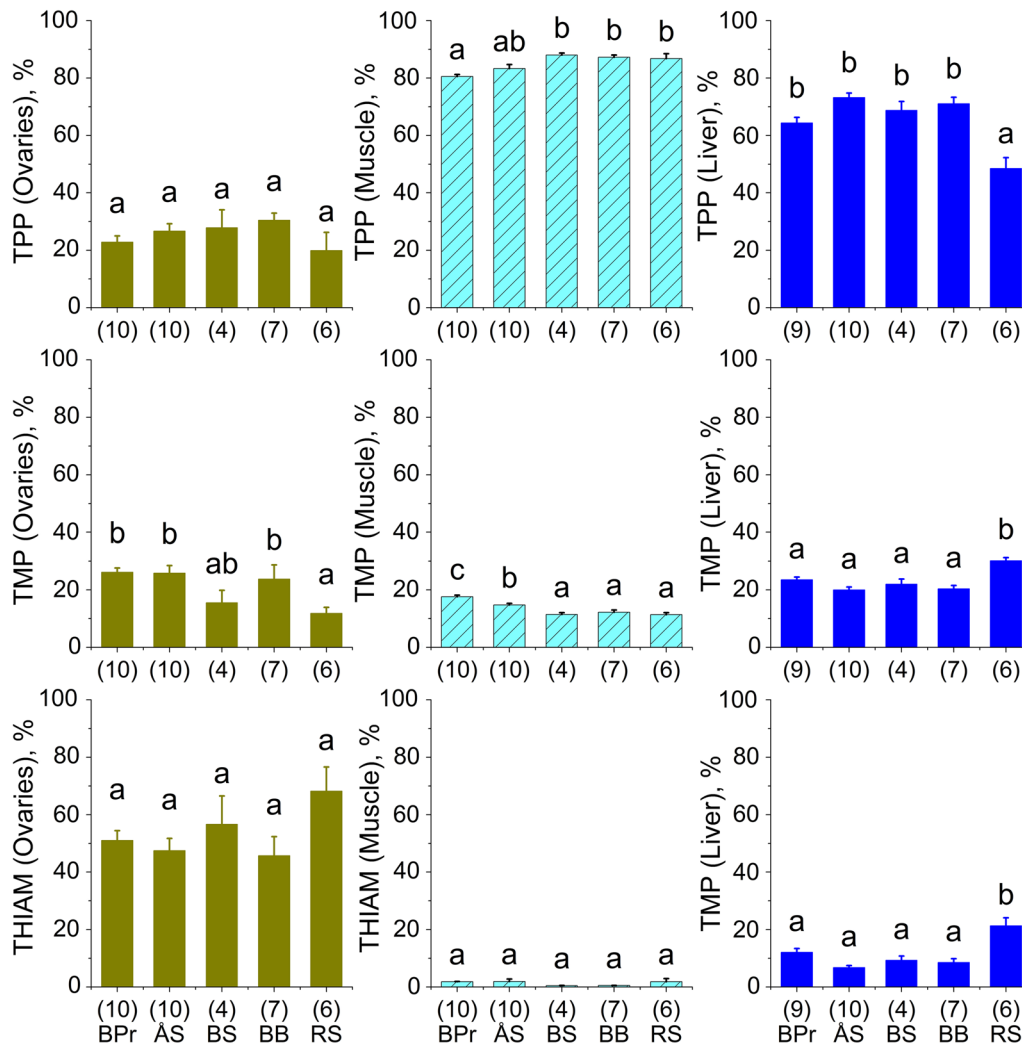
#### Carotenoid concentration in muscle

The muscular concentration of total carotenoids was significantly lower in the spawning salmon than in the salmon caught during the sea-phase of the spawning



**Fig. 5** Relationship of the total thiamine (TotTHIA) concentration of the liver, ovaries, and muscle with the distance of the spawning run. Regressions, with 95% confidence limits, are indicated for salmon on their spawning run from the Baltic Proper (0 km) to the River Simojoki (1400 km) including the sea phase and the salmon at spawning. Note the difference of scales in the y-axis of the panels



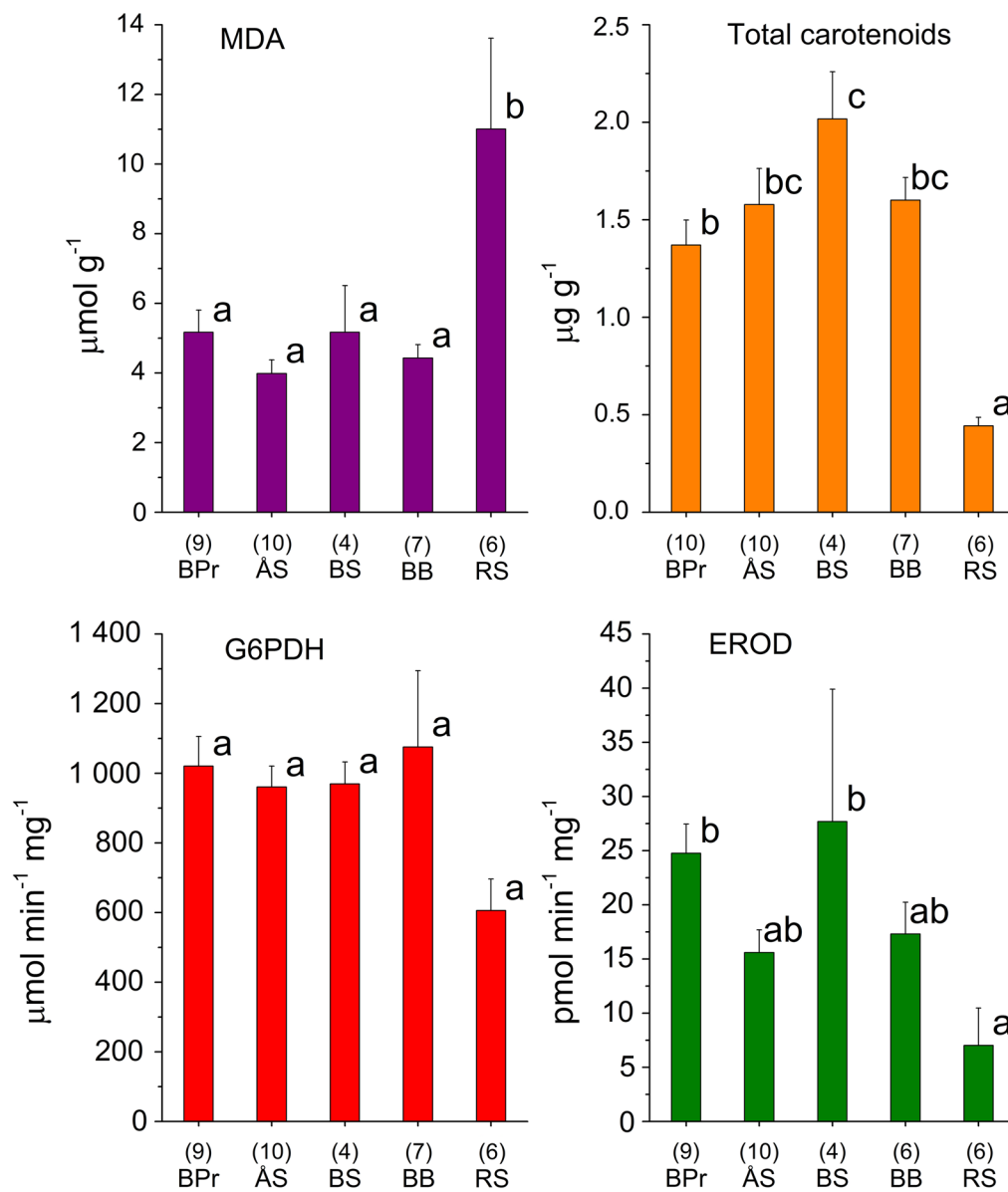


**Fig. 6** Mean (+SE) proportions of thiamine components (thiamine pyrophosphate, TPP, thiamine monophosphate, TMP, and free thiamine, THIAM) of total thiamine in ovaries, liver and muscle of 2-sea-year-old salmon at different sampling locations (see legend in Fig. 1). Significant differences ( $p < 0.05$ , post hoc SNK in one-way ANOVA) in the means between the sampling locations are indicated with no common letter. The number of specimens is given in parentheses

run, and the concentration was the highest in the salmon from the Bothnian Sea (Fig. 7). At spawning, the concentration of total carotenoids was one third of that in the Baltic Proper salmon and one fourth compared to the Bothnian Sea salmon. However, there was no linear relationship between the sampling locations during the spawning run of the salmon and their muscular concentration of total carotenoids (not shown). In addition, the carotenoid concentration of salmon muscle did not show any relationships with the concentration of TotTHIA of the ovaries, the liver, or muscle (not shown).

#### Malondialdehyde concentration and enzyme activities in the liver

The hepatic MDA concentration of the spawning salmon was significantly higher (double) than the concentrations for the salmon caught in the sea during the spawning run (Fig. 7). Although the hepatic G6PDH activity was the lowest at spawning, the differences between the salmon from the different locations were not significant (Fig. 7). However, when comparing the spawning females that had been feeding in the Baltic Proper with those caught from the Baltic Proper the difference in the G6PDH activity was significant



**Fig. 7** Concentrations of malondialdehyde (MDA) in the liver and total carotenoids in muscle and hepatic activities of glucose-6-phosphate dehydrogenase (G6PDH) and 7-ethoxyresorufin-O-deethylase (EROD) of 2-sea-year-old salmon during the spawning run (see legend in Fig. 1). Significant differences ( $p < 0.05$ , post hoc SNK in one-way ANOVA) in the means between the sampling locations are indicated with no common letter. The number of specimens is given in parentheses

( $p < 0.05$ ). The hepatic EROD activity was lowest in the spawning salmon, but was not significantly different from that in salmon from the Åland Sea and Bothnian Bay (Fig. 7). The EROD activity of the spawning salmon that had been feeding in the Baltic Proper was only 27% of that of the salmon captured from the Baltic Proper.

#### Total differences of salmon between the sampling places

The salmon from the Baltic Proper and spawning salmon in the River Simojoki differed significantly from each other and also from the salmon from all other locations as indicated by the SIMCA classification (Fig. 8). In the PCA, using all the biochemical measures as loadings (total lipid content, the proportions of



## Discussion

### Changes in lipid and fatty acid content

The average lipid content in the muscle of the salmon at the beginning of the spawning run from the Baltic Proper in the spring tended to be lower than in the muscle of salmon that had been caught from the same area during their feeding migration in the late autumn (8.7%; Keinänen et al., unpubl.). The lipid content of prey fish decreases by the spring [1, 7, 67, 80], and the feeding activity of salmon decreases at the same time [13]. The salmon from the present study that were caught in the spring had probably already used part of their lipid stores acquired during high autumn feeding activity.

Although the muscular lipid content did not differ between the sampling locations in the sea, it halved by spawning time. This decrease was in the same range as reported for salmonine species during the pre-spawning period in general [6]. In 1–3 sea-year salmon, even a decrease of 60% in muscle lipid content was found from the start of the spawning run in the Baltic Proper till the spawning in the River Simojoki [18]. In the present study's spawning salmon, the muscle lipid content ( $3.65 \pm 0.78\%$ ) was much lower than in those salmon sampled at spawning in 1991 and 1992 (means of 7.58 and 9.45% respectively) [81], i.e., in the early years of the highest incidence of M74 [4, 11].

The activity of hepatic G6PDH, which is known to decrease with increasing dietary lipids [82–84], did not differ significantly between salmon caught at all locations. However, in those spawning salmon that had been feeding in the Baltic Proper the G6PDH activity was approximately 50% and significantly lower than in salmon captured from the Baltic Proper, probably due to the mobilisation of FAs from body lipids for metabolism, the development of oocytes, and various kinds of physical activity. Thus, lipids from body stores probably affected hepatic G6PDH activity similarly to how dietary lipids would have.

Among the dominant FAs, the proportions, i.e. relative amounts, of 14:0, 16:0, and thus that of SFAs, decreased most during the spawning run. Their proportions in salmon in the Baltic Proper were nearly as large as in salmon caught at the same location during their late autumn feeding migration [4]. During the spawning run, the proportions of 14:0, 16:0, and total SFAs were already significantly lower in salmon from the last sampling location in the sea in the Bothnian Bay than in the Baltic Proper. As all the sampled fish were females, SFAs were also used for the production of oocytes. According to Tocher [36], both SFAs and MUFAs, usually present in high percentages in storage triacylglycerols (TAGs), are heavily catabolised for energy, particularly in pre-spawning fish. In the present study, the decreases in the

proportions of 18:1*n*-9 and total MUFAs were not prominent, probably because 18:1*n*-9 is an important constituent in both storage fat and structural phospholipids [36]. Thus, the utilisation of neutral lipids with a consequent relative increase in phospholipids did not greatly affect the 18:1*n*-9 proportion. All individual HUFAs were conserved more than other FAs, because they are characteristic of phospholipids. The consequent increase in the proportions of *n*-3 PUFAs and total PUFAs was greatly brought on by the quantitatively important DHA, which is a crucial component of the phospholipids of cellular membranes and is not easily used for energy production [36].

The increase in the proportion of DHA and *n*-3 HUFAs towards the spawning period is a general response among fish [36]. The proportion of DHA, contrary to its concentration, also increases during deprivation of food, as reported for sprat and herring [7]. In these species, the DHA proportions were higher in spring than in autumn and higher in the leaner specimens of the Gulf of Finland compared to the other Baltic Sea basins [7]. Moreover, HUFAs are the desaturation and elongation products of shorter PUFA precursors, although those enzyme activities are considered negligible in Atlantic salmon receiving HUFAs from their diet [36]. It is possible that the increase in the proportion of DHA partly results from some synthetic activity compensating for the cessation of dietary supply in pre-spawning fish [85], because DHA is still required for the synthesis of vitellogenin during the pre-spawning period [86]. However, considering the concentrations, i.e., the actual amounts, of FAs in muscle, they all decreased considerably by spawning time.

A major role of lipids in fish is to store energy as FAs and provide metabolic energy in the form of ATP through  $\beta$ -oxidation of FAs [36]. In feeding salmonine species, lipids largely accumulate in muscle tissue and in the body cavity as a visceral depot, the stores of which contain *n*-3 PUFAs in large percentages [6]. Although the proportion of PUFAs had increased by spawning, their actual amount had decreased by 46%, along with the use of lipids for metabolism and oocyte formation, during the spawning run and pre-spawning fasting. The hepatic concentration of MDA increased simultaneously, indicating large-scale peroxidation of PUFAs [63, 64]. Calculated from a linear model of the present study's data, an increase of 1% in the muscular PUFA proportion of salmon was associated with an increase of  $1.6 \mu\text{mol g}^{-1}$  in hepatic MDA concentration. In yolk-sac fry, the accumulation of MDA has been linked to M74 mortalities [87]. However, this has been linked to increased concentrations of DHA and *n*-3 PUFAs provided by females and an increased rate of lipid peroxidation [30, 56]. Since the MDA concentrations of the salmon at spawning were

approximately double the concentrations detected in the salmon during the earlier phases of the spawning run, the oxidative stress caused by PUFA peroxidation was severe, although not fatal.

#### Manifestation of energy metabolism in total thiamine content of tissues

Although thiamine is water soluble, its halftime in non-breeding salmonines is fairly long. More than 10% of the original thiamine dose injected (i.p.) in juvenile steelhead trout [*Oncorhynchus mykiss* (Walbaum)] was detected in their muscle after six months [88]. In the present study's salmon, from a year with an insignificant incidence of M74 [4], no clear trend in the concentration of the TotTHIA of muscle throughout the spawning migration was detected, although the concentrations at spawning were the smallest. Similarly, Karlsson et al. [89] found no decrease in the muscular thiamine concentration in the mid-1990s, when the incidence of M74 was high [4].

In hatchery salmon, the concentration of TotTHIA at spawning was, as in the present study, also largest in the liver; more than half this concentration was detected in the muscle, and one tenth in the blood [28]. In the present study, the decrease in the concentration of TotTHIA until spawning was clearer in the liver (>60%) than in the other tissues studied, as in the study by Karlsson et al. [89], although their low sample number prevented statistical comparisons. In a year (1995) with high M74 mortality, Koski et al. [28] detected lower concentrations of total thiamine (3.3–6.6 nmol g<sup>-1</sup>) in the liver of spawning female salmon from the River Simojoki than we found for the salmon liver in the present study (6.3–10.3 nmol g<sup>-1</sup>). However, the values are probably not strictly comparable due to a different analysis method.

Although no significant decrease was detected in the concentration of TotTHIA in the muscle, contrary to the liver and ovaries, during the spawning run in the present study, the change in both the hepatic and muscular TotTHIA concentration was related to the decrease in the TotTHIA concentration of the eggs. In the single M74 female from the River Simojoki, whose offspring all died (while for all other females, offspring survival was >94%), the TotTHIA concentration in both the eggs and muscle was the lowest measured in the present study. In a study by Amcoff et al. [14], the concentrations of thiamine in both ovaries and muscle were lower in those salmon whose offspring displayed M74 symptoms than in those females that produced normal yolk-sac fry.

Although no M74 mortalities were detected among offspring of salmon that had been feeding in the Baltic Proper, the concentration of TotTHIA in the ovaries of salmon decreased significantly during the sea phase of the spawning migration, and the ovulated eggs had only

a quarter of the TotTHIA concentration of the ovaries from salmon sampled in the Baltic Proper. In the severe M74 years (1995–1997), caused by abundant feeding on young sprat in the Baltic Proper [4], the concentrations of thiamine in salmon liver and gonads tended to be lower in May than in April [89]. However, it is not very likely that the decrease in the concentrations of TotTHIA in the salmon liver and ovaries of the present study would have been clearer if the salmon from the Baltic Proper had been sampled earlier in the spring. In the salmon caught during the feeding migration in the same area in late autumn [Keinänen et al., unpubl.], the mean hepatic TotTHIA was only slightly larger than in the salmon from the present study.

Thiamine concentrations diminished as salmon decreased their food consumption and finally ceased feeding. Salmon had stopped feeding in the Bothnian Sea or at the latest when leaving it, approximately 500 km before entering the spawning river [18]. In addition, pre-spawning salmon fast until spawning in late October. The fasting period therefore lasts for about four months [18]. The more metabolic energy stored in lipids (as FAs need to be mobilised during the spawning run and pre-spawning fasting for the metabolic activities, such as acclimation to river water, the effort expended in swimming to the river's spawning areas, and the ripening of oocytes), the more thiamine is depleted, because thiamine is required for energy metabolism [38]. However, high body lipid, and thus PUFA content of fish in general, has also been negatively correlated with thiamine concentrations during the feeding phase [7, 39].

Specifically, HUFAs, which are very susceptible to peroxidation [45, 46], cause an additional decrease in thiamine [51, 52]. Thiamine functions as a site-specific antioxidant in peroxidation reactions, in which it is converted to thiochrome and other forms, which can no longer act as the co-enzyme [47, 51, 52] and are excreted via the kidneys [38]. This process probably explains why the TotTHIA concentration decreased as a function of the increase in the concentration of DHA in sprat and herring and was lowest in the youngest specimens, in which the PUFA content was the highest [7]. As DHA, which among the FAs contains the highest number of double bonds, was the dominant PUFA during the spawning run, similarly to salmon during feeding migration [4], it was largely responsible for the depletion of thiamine in peroxidation reactions. According to a linear model, an increase of 1 mg g<sup>-1</sup> in the concentration of DHA in the muscle resulted in a decrease of 2.4 nmol g<sup>-1</sup> in the concentration of hepatic TotTHIA in the salmon sampled during the feeding migration in the autumn (Keinänen et al., unpubl.). Although oxidation of 18:1n-9 also increases oxidative stress, against which thiamine



also acts as an antioxidant [47], the primary source of oxidative stress in fish tissues is peroxidation of HUFAs [45, 46]. As the content of *n*-3 HUFAs increases with the increase in the lipid content of fish, the tissues of the fattest fish are most prone to lipid peroxidation [6, 43, 44] and thiamine deficiency.

#### Thiamine component composition in relation to lipid content

Although the TotTHIA concentration was highest in the liver, the thiamine of salmon is principally located in the muscle tissue, which is considerably more voluminous than the liver or ovaries. However, most (80–88%) of the TotTHIA in the muscle of salmon on their spawning run was detected as the co-enzyme form (TPP), similarly as has in general been detected for vertebrate soft tissues such as muscle, the brain, and the liver [38]. Muscular TPP probably does not easily supply additional thiamine for transporting to oocytes, because >90% of the mitochondrial TPP in muscle is bound to enzymes [52]. The proportion of muscular TPP was higher in salmon after a decrease in feeding than in salmon caught at the first location of the spawning run in the Baltic Proper, and the TPP proportion increased as a function of the whole body lipid content of sprat and herring [7]. Apparently, in all these salmon, most of the thiamine was connected to metabolic processes as the TPP derivative. Muscular lipids were probably intensively turned over to metabolic energy and for the development of oocytes during pre-spawning fasting.

The median THIAM concentration in the muscle of salmon on the spawning run was very small, 0.070 nmol g<sup>-1</sup>, because most of the thiamine existed as TPP. The proportion of THIAM in muscle was 0.3–9.4%, which was lower than in most salmon caught during the feeding period in the autumn, except in the few fattest ones among these salmon [Keinänen et al., unpubl.]. In the spawning salmon, the median concentration of THIAM in muscle was only 0.036 nmol g<sup>-1</sup>. The smallest concentration of THIAM in muscle and eggs was detected in the single M74 female from the River Simojoki. Being very small, the hepatic THIAM concentration changed very little during the spawning run, due to which it could not be used as an indication of thiamine status, contrary to the hepatic TotTHIA concentration.

#### M74 mortality and biochemical indices

Thiamine deficiency is manifested more easily in the yolk-sac fry than in their parents, because offspring, first as embryos and after hatching as yolk-sac fry, must rely on the thiamine reserves of yolk provided by the female parent for more than half a year during the rapid cell division and growth phase. In cases when adult salmon

have a lower supply of thiamine than required for energy metabolism, taking into account thiamine depletion, adult salmon also suffer from thiamine deficiency. According to the Finnish M74 monitoring, all offspring of the females showing wriggling behaviour died due to thiamine deficiency [11].

All the offspring of one female in the present study succumbed to M74, whereas no M74 mortalities were recorded among the offspring of the five other females. The mean THIAM concentration (2.05 ± 0.22 nmol g<sup>-1</sup>) in the eggs from the present study's River Simojoki salmon, excluding the single M74 female, was clearly larger than the limit value for any possible M74 occurrence. On the basis of long-term Finnish M74 monitoring [4, 11], at an egg THIAM concentration of ≤ 0.2 nmol g<sup>-1</sup>, yolk-sac fry mortality of 100% is expected, and at a concentration of ≤ 0.5 nmol g<sup>-1</sup>, the female is very probably a M74 female, whereas at the THIAM concentration of eggs ≥ 1.0 nmol g<sup>-1</sup>, possible mortalities are not caused by thiamine deficiency. The mean THIAM concentration in the eggs of the non-M74 females in the River Simojoki from the present study was also considerably larger than the median concentration of 0.35 nmol g<sup>-1</sup> in the eggs of the salmon included in the Finnish M74 monitoring in 1994–1995, when the incidence of M74 was very high [4]. However, due to the variation in the feeding areas of salmon, and thus in their dietary composition and abundance [1, 3, 33], the egg THIAM concentrations of individual salmon included in the Finnish M74 monitoring have varied greatly in some years, even more than a 100-fold in a year [4]. In four salmonine species from Lake Ontario, the LC50 values of free thiamine in their eggs varied from 0.26 to 1.32 nmol g<sup>-1</sup> [2]. Although the mean THIAM concentrations of eggs in this study's non-M74 females had not decreased alarmingly when metabolising lipids and peroxidation-susceptible PUFAs, the salmon may have been physiologically stressed, as has been reported for humans and other vertebrates with a reduced thiamine status [52].

In addition to the decreased concentrations of TotTHIA and its components in the liver and ovaries by spawning, the risk of developing oxidative stress also became apparent in these spawning salmon in their reduced muscular carotenoid concentrations. Carotenoids have also been transported from the muscle into developing oocytes during exogenous vitellogenesis. Small carotenoid concentrations in the eggs of M74 female and a link between M74 and oxidative stress have been found in earlier studies [29, 30, 56] and in the Finnish M74 monitoring [11]. The smallest carotenoid concentrations in the muscle were detected in the single M74 female and in one non-M74 female. Thiamine deficiency in itself is known to increase oxidative stress [51, 90],

and carotenoids as antioxidants are therefore consumed. Contrary to the thiamine concentration, no decrease in the carotenoid concentration was found during the marine phase of the spawning run. In addition, the carotenoid concentration had no significant relationships with the concentrations of TotTHIA in any tissue studied during the spawning run. As carotenoids are powerful general antioxidants [59, 91], they are depleted due to oxidative stress caused by various factors (e.g. environmental toxicants [92]), whereas thiamine functions as an antioxidant against lipid peroxidation products [47, 51, 52]. The muscular carotenoid concentrations appeared to vary greatly among the different sampling locations during the spawning run. As salmon obtain astaxanthin from the crustaceans in the alimentary tract of sprat and herring [93], the dietary condition of prey fish directly affects salmon astaxanthin concentration. In their turn, the astaxanthin production by Baltic Sea mesozooplankton is affected by changes in environmental temperature and salinity, for example [94].

During the spawning run, there was a large variation in hepatic EROD activity, but in spawning salmon, the EROD activity was significantly lower than in salmon caught in the sea. This was unexpected, because organohalogenes are known to induce EROD activity [71], and the concentrations of organohalogenes in the muscle of the present study's salmon had increased by spawning [18]. The salmon's organohalogen burden had barely changed, but as a consequence of hydrolysing body lipids for metabolism and transferring them to the developing oocytes, the xenobiotic concentration per body mass increased [18]. However, it was reported that the concentrations of PCBs, DDTs, and polybrominated diphenyl ethers did not differ between the M74 females and non-M74 females [95]. Likewise, the thiamine deficiency of lake trout [*Salvelinus namaycush* (Walbaum)] in the Great Lakes had no relationship with the concentrations of organochlorines in their eggs [96]. However, oestrogens, whose concentrations maximise before spawning, are known to suppress EROD activity [97, 98], and some PUFAs (e.g. DHA) have also been demonstrated to suppress it [99]. Vitellogenin, which is synthesised in the liver, consists of approximately 20% of DHA [86]. Thus, high PUFA contents may have for their part impaired hepatic EROD functioning in metabolising oestrogens and organohalogenes, the latter of which were translocated in the turnover of tissue energy stores during pre-spawning fasting.

#### **Thiamine status of salmon in relation to their feeding conditions**

It is possible that the salmon caught in the different sampling locations included individuals from rivers that do

not run into the Bothnian Bay. However, all the salmon sampled at spawning had apparently returned to their home river, because they were caught at the mouth of the River Simojoki. In any case, most of the sampled salmon had been feeding in the Baltic Proper. The only exceptions were one salmon from the Bothnian Bay and the single M74 female which ascended the River Simojoki, both of which had probably been feeding in the Bothnian Sea. In all the other salmon, the ratio of the total concentration of PCDD/F to the concentration of CoPCB and the muscle FA signatures were characteristic of those salmon feeding in the Baltic Proper [4, 18, 66].

In the Baltic Proper, salmon mainly prey on sprat [3, 17], which was also found by analysing the stomach content of the present study's salmon [18]. Those salmon caught along the route of the spawning run may have been feeding in various sub-areas of the Baltic Proper, which differ in relation to the proportions of sprat and herring in a salmon prey biomass [3, 33]. This caused the variation seen in the THIAM concentrations in the eggs of non-M74 females, for example. According to Jacobson et al. [33], the dietary proportions and size of sprat and herring also appeared to depend on the size of salmon, but in the present study, all the salmon had spent two years in the sea and were of the same size.

Salmon prefer small prey [17, 18]. As herring grow fastest in the southernmost parts of the Baltic Sea, only the youngest age groups of herring in the area are of the right size to be preyed on by salmon [3]. In contrast, all sprat are of a suitable size for salmon [3]. In those years with a high or moderate incidence of M74, mortalities were regarded as a consequence of feeding abundantly on young sprat in the Baltic Proper [1, 3]. Feeding principally on sprat in the southern Baltic in 1998, when there was a moderate incidence of M74, was also verified by FA signature analysis [4].

Between 2002 and 2004, when the salmon from the present study were feeding in the Baltic Proper, the sprat stock had declined to its smallest size after its explosive increase at the turn of the 1990s [11], and the year classes for sprat in these years were especially small [100]. All the River Simojoki salmon at spawning addressed in this study (excluding the single M74 female) had been feeding in the Baltic Proper and mainly on sprat, indicated by the high 18:1*n*-9/ARA and 14:0/ARA ratios [4]. Apparently, these salmon had largely fed on older sprat specimens whose lipid content was lower, and whose thiamine concentration was higher than in young sprat [1, 7], because the THIAM concentrations in their eggs were higher than the estimated limit value for M74 symptoms. However, the THIAM concentration in eggs from the River Simojoki salmon had decreased to half the THIAM concentration in the ovaries of salmon from

the Baltic Proper. Consequently, their thiamine resources had depleted during the spawning run and pre-spawning fasting, but to a lesser extent than in those cases that had resulted in M74.

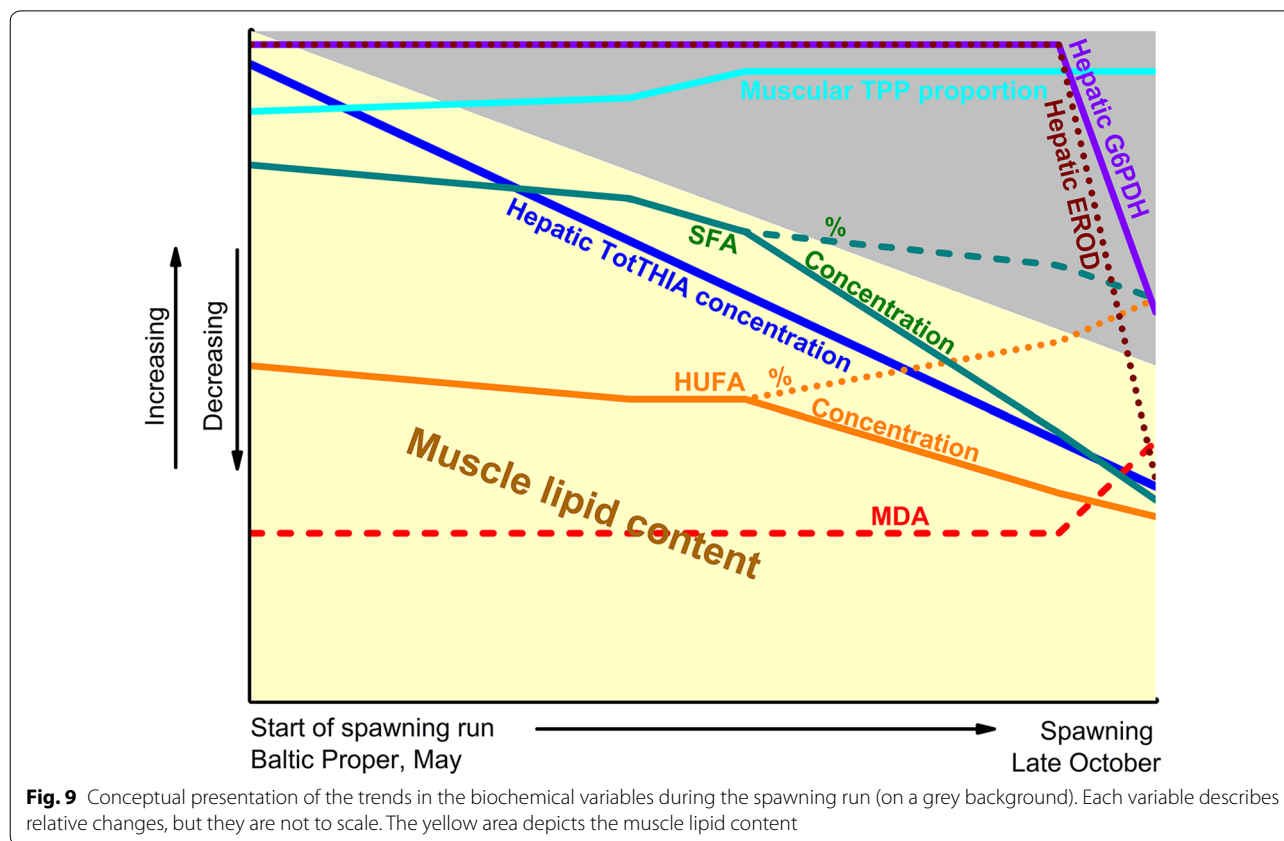
Because thiamine concentrations decreased after the salmon diminished and ceased feeding, the thiaminase of prey fish cannot be the cause of thiamine deficiency in salmon eggs and tissues, contrary to an old and long-lived hypothesis, as has already been demonstrated in detail by Mikkonen et al. [3] and Keinänen et al. [1]. As discussed by Keinänen et al. [4], the large size and high growth rate of salmonines whose offspring suffer from thiamine deficiency also verifies that the thiamine supply from prey fish has been high enough to enable proper growth. The growth rate of salmon has been proper, ranging from 20 to 32 cm per year from the first to second growth seasons during the period 1971–2006 [1, 3]. Growth was faster during the moderate or high incidence of M74 in the 1990s and in the 2000s than that from the mid-1970s to the early 1980s, when M74 mortalities were insignificant [3, 26]. As retarded growth is a symptom of dietary thiamine deficiency [38], the thiamine reserves in salmon at the start of the spawning run in the Baltic Proper, even during the years of high M74 incidence, have apparently been adequate considering the requirement for their proper growth [40]. Indeed, there are no publications that could have proven the linkage of thiaminase in prey fish to thiamine deficiency in salmonines, despite the fact that the topic has been greatly studied, with dozens of publications concerning it. By contrast, an increased requirement of thiamine with an increase in the dietary energy content is well known for humans, as well as for fish [40, 41]. Likewise, the depletion of thiamine in energy metabolism and peroxidation reactions in the tissues of vertebrates is well documented [41, 51, 52]. In all our studies concerning M74 and in our reviews of publications concerning other thiamine deficiency syndromes of salmonines (e.g. TDC), thiamine deficiency is consistent with such a cause [1, 3, 4, 7]. As the accumulation of lipids and *n*-3 PUFAs in fatty fish species increases with an increase in the lipid and *n*-3 PUFA content of a fish-based diet [6, 44], extreme circumstances can result in serious thiamine deficiency in salmonines, as was found in the years with the highest incidence of M74 during the 1990s [4, 11].

The proportion of sprat in general decreases from the southern to the northern Baltic, meaning that in the Bothnian Sea, herring is the dominant species [17, 26]. Sprat shoals, however, disperse from their spawning areas of the Baltic Proper, particularly when the sprat stock has increased following the years of their very successful reproduction [32]. Practically no sprat have been caught from the most northern part of the Gulf of Bothnia, the

Bothnian Bay [100]. Between 2002 and 2003, age 0–1 herring were abundant as prey for salmon in the Bothnian Sea, as these year classes were very strong [100]. Age 0 herring during the late summer already attain lengths that are appropriate for them to be prey for salmon [17, 101]. In the Bothnian Sea, only approximately 14% of the salmon from the River Simojoki stock halt to feed [34], based on a long-term average. The concentration of DHA and total PUFAs in the youngest specimens of herring, similarly to sprat, are the highest [7]. Moreover, the proportion of PUFAs was or tended to be on average higher in herring than in sprat [7, 80]. In salmon feeding between 2002 and 2003 in the Bothnian Sea, the proportion of DHA, and consequently that of *n*-3 PUFAs and total PUFAs, was higher than in salmon caught from the Baltic Proper [4]. Thus, it is possible that PUFAs obtained by feeding on young fatty herring in abundance enabled the sporadic development of M74 in a year when feeding in the Baltic Proper did not result in thiamine deficiency [4].

## Conclusions

The thiamine resources of salmon clearly decrease during their spawning run, even in those years for which no M74 mortalities relating to feeding extensively on young sprat in the Baltic Proper have been recorded. The obvious and decisive reason for the decrease in the concentration of TotTHIA in the liver and ovaries through the spawning run, along with its consumption in general cell metabolism, is lipid peroxidation, which appeared as an increased hepatic concentration of the lipid peroxidation product MDA. The concentration of carotenoids decreased only during the pre-spawning fasting, indicating increased oxidative stress caused by lipid peroxidation and a thiamine deficiency spiral. These results are consistent with our earlier results, which concluded that the cause of the thiamine deficiency of salmonines is especially an excess dietary supply of HUFAs, of which more than half is DHA [1, 4, 7]. The body composition of salmon changes considerably during the spawning run, which is also reflected in the different hepatic biochemical indices; in terms of biochemical composition, the salmon at the start of the spawning run and the salmon at spawning were characterised separately from each other and from the salmon along the spawning route. The decrease in the total lipid content along the spawning run and the accompanying changes in fatty acid composition were marked. The proportion of SFAs, particularly that of 16:0, decreased the most, indicating their intense use for metabolic energy and the formation of oocytes. By contrast, the proportions of all individual HUFAs increased, despite lower concentrations. As the decrease in the concentrations of TotTHIA and its components were



detected during the sea phase of the spawning run, the thiamine status of the ascending salmon might already be determined when they enter the river mouth. Significant changes in the other biomarkers (total carotenoids, MDA, G6PDH, and EROD) were detected only in the spawning salmon, and they therefore cannot be used to describe the salmon's status earlier in the spawning run. The main schematic trends in the biochemical variables during the spawning run are summarised in Fig. 9.

### Supplementary information

**Supplementary information** accompanies this paper at <https://doi.org/10.1186/s10152-020-00542-9>.

**Additional file 1.** The identified fatty acids (FAs) with the abbreviation and common names.

**Additional file 2.** Proportions (a) and concentrations (b) of fatty acids in the muscle of 2-sea-year-old salmon during the 1400 km spawning run from the Baltic Proper through the Gulf of Bothnia to the north-eastern Bothnian Bay at four locations and at spawning time in the River Simojoki.

**Additional file 3.** Relationship of the total thiamine (TotTHIA) concentration of ovaries to that of (a) the liver and (b) muscle.

### Acknowledgements

We thank the fishermen and the staff from the Natural Resources Institute Finland (formerly the Finnish Game and Fisheries Research Institute) for the sampling of salmon.

### Authors' contributions

PJV and MK designed the study. TP organised sampling and MR, TR and SN were responsible for biochemical analyses. PJV and RK did the statistical calculations, and PJV drew the graphics. All authors contributed to data interpretation. PJV and MK wrote the manuscript, with significant input from all the co-authors. All authors read and approved the final manuscript.

### Funding

This research was partly funded by the Nordic Council of Ministers and the Academy of Finland (project DIOXMODE, no. 102557, in the Baltic Sea Research Programme BIREME).

### Availability of data and materials

The data can be found at [luke.fi/radar](http://luke.fi/radar) (keyword: spawning run salmon).

### Ethics approval and consent to participate

The research involved no human subjects, human material, or human data. The fish used were taken from the commercial catches of professional fishermen, and were therefore not subject to national legislation concerning animal experimentation.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

### Author details

<sup>1</sup> Natural Resources Institute Finland (Luke), P.O. Box 2, 00791 Helsinki, Finland. <sup>2</sup> Faculty of Biological and Environmental Sciences, Molecular and Integrative Biosciences, University of Helsinki, P.O. Box 65, 00014 University of Helsinki Helsinki, Finland. <sup>3</sup> Finnish Food Authority, P.O. Box 200, 00027 Ruokavirasto, Finland. <sup>4</sup> Helsinki University Lipidomics Unit (HiLIPID), Helsinki Institute of Life Sciences (HiLIFE), and Molecular and Integrative Biosciences Research

Programme, Faculty of Biological and Environmental Sciences, University of Helsinki, P.O. Box 65, 00014 University of Helsinki Helsinki, Finland.

Received: 14 January 2020 Accepted: 11 August 2020

Published online: 28 August 2020

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