Author(s): Vuorinen, Pekka J.; Juntunen, Esa-Pekka; Iivari, Juha; Koski, Perttu; Nikonen, Soili; Rokka, Mervi; Ritvanen, Tiina; Pakkala, Jukka; Heinimaa, Petri; Keinänen, Marja

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Lipid-related thiamine deficiency cause mortality of river lampreys (*Lampetra fluviatilis*) during pre-spawning fasting

Pekka J. Vuorinen [a,b,c,*], Esa-Pekka Juntunen [d], Juha Iivari [d], Perttu Koski [e], Soili Nikonen [f], Mervi Rokka [f], Tiina Ritvanen [f], Jukka Pakkala [g], Petri Heinimaa [h], Marja Keinänen [a]

[a] *Natural Resources Institute Finland (Luke), Natural Resources, Fisheries and Fish Resources, P.O. Box 2, FI-00791 Helsinki, Finland*
[b] *University of Helsinki, Faculty of Biological and Environmental Sciences, P.O. Box 65, FI-00014 Helsinki, Finland*
[c] *Department of Biological and Environmental Science, University of Jyväskylä, P.O. Box 35, FI-40014 Jyväskylä, Finland*
[d] *Natural Resources Institute Finland (Luke), Laivurintie 6, FI-94450 Keminmaa, Finland*
[e] *Finnish Food Authority, Animal Health Diagnostic Unit, Elektroniikkatie 3, FI-90590 Oulu, Finland*
[f] *Finnish Food Authority, Laboratory and Research Division, Chemistry Unit, P.O. Box 200, FI-00027 Ruokavirasto, Finland*
[g] *Centre for Economic Development, Transport and the Environment, South Ostrobothnia Office, P.O. Box 77, FI-67101 Kokkola, Finland*
[h] *Natural Resources Institute Finland (Luke), Survontie 9, FI-40500 Jyväskylä, Finland*

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ABSTRACT

River lampreys (*Lampetra fluviatilis*) were caught in the fall 2014 on entering the River Perhonjoki for spawning and kept at a hatchery until spawning in late spring 2015 to produce larvae for compensatory stockings. Since the lampreys died massively from early February onwards, they were investigated in March and May to clarify the cause of the deaths. The symptoms in lampreys resembled those of lipid-related thiamine (vitamin B1) deficiency of salmonines, called the M74 syndrome in the Baltic Sea area. Because the lipid content of lampreys was known to be high, thiamine concentrations were analyzed in the liver and ovulated unfertilized eggs, and the mass, length, and whole-body lipid content were also measured. The hepatic total thiamine (TotTh) concentration was significantly negatively correlated with the body lipid content and fatness index (mass to length ratio) in both females and males. In females, the hepatic TotTh concentration was less than half that in males, and the most moribund lampreys were the largest and fattiest females. Females that survived until artificial stripping of the eggs were smaller, and their hepatic thiamine concentration was higher, and the fatness index lower, than in females in March. The concentration of free thiamine in the eggs had a stronger positive correlation with the hepatic TotTh concentration than the phosphorylated thiamine derivatives and was also significantly and negatively correlated with the fatness index and mass. It was concluded that increased lipid peroxidation due to the mobilization of polyunsaturated fatty acids from lipids in exogenous vitellogenesis consumed thiamine as an antioxidant during pre-spawning fasting—especially in the fattiest females—and thus caused their death. It is suggested that to ensure compensatory stockings, the largest lampreys could be thiamine-injected at the hatchery to improve their survival until stripping of the eggs and to improve the eggs' thiamine status.

1. Introduction

After several years spent in river sediments and metamorphosis, river lampreys (*Lampetra fluviatilis*) (hereafter, lampreys) migrate from the river to the sea in the spring (Aronsuu, 2015). After 1–2 summers (Ojutkangas and Valtonen, 1998; Aronsuu et al., 2015), they return to the rivers in the early fall for spawning, which takes place the following May. Most lamprey populations are skewed towards a higher male to female ratio (Bartel et al., 2011).

Many stocks of lampreys have suffered from overfishing, pollution, and habitat destruction, and further, dams have prevented their entry into spawning grounds (Aronsuu, 2015; Maitland et al., 2015; Almeida et al., 2021). Lampreys have therefore been caught in the fall when they enter the rivers for compensatory production of larvae in hatcheries and to stock them (Kujawa et al., 2018; Moser et al., 2019). From the River Perhonjoki, which flows into the Gulf of Bothnia in the Baltic Sea, lampreys have been caught for compensatory purposes in the early fall since

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* Corresponding author at: University of Helsinki, Faculty of Biological and Environmental Sciences, P.O. Box 65, FI-00014 Helsinki, Finland.
E-mail addresses: pekka.vuorinen@helsinki.fi, pekka.j.vuorinen@jyu.fi, pekka.vuorinen@gmail.com (P.J. Vuorinen), pekka.juntunen@luke.fi (E.-P. Juntunen), juhaiivari52@gmail.com (J. Iivari), perttu.koski@fimnet.fi (P. Koski), soili.nikonen@ruokavirasto.fi (S. Nikonen), mervi.rokka@ruokavirasto.fi (M. Rokka), tiina.ritvanen@ruokavirasto.fi (T. Ritvanen), jukka.pakkala@ely-keskus.fi (J. Pakkala), petri.heinimaa@luke.fi (P. Heinimaa), m.e.keinanen@gmail.com (M. Keinänen).

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1985 by the South Ostrobothnia Office of the Centres for Economic Development, Transport and the Environment (ELY Centre South Ostrobothnia), which has kept lampreys and incubated eggs at the hatchery of the Korpela hydroelectric power plant on the nearby River Lestijoki. In 2008, this activity was moved to the Keminmaa hatchery (at the mouth of the River Kemijoki) of the Finnish Game and Fisheries Research Institute (since January 1, 2015, the Natural Resources Institute Finland, Luke).

In lampreys caught in the fall 2014, elevated and increasing prevalence of mortality occurred at the hatchery since early February 2015 toward the spawning period. Before dying, they showed similar symptoms (including uncoordinated swimming) as those observed in adult salmon (*Salmo salar*) and their offspring, yolk-sac fry [i.e., free embryos or eleutheroembryos (Balon, 1975)] suffering from thiamine (vitamin B1) deficiency, called the M74 syndrome in the Baltic Sea area (Bylund and Lerche, 1995; Koski et al., 2001; Keinänen et al., 2012; Vuorinen et al., 2021). These lampreys easily became tired and passive, and they therefore sank to the bottom of the rearing tanks like thiamine-deficient salmon yolk-sac fry and adults (Keinänen et al., 2000, 2008; Vuorinen et al., 2014a). The dead lampreys often remained coiled. These symptoms and mortality were seen in the largest lampreys, while the smallest specimens appeared normal. Eel (*Anguilla japonica*) experimentally fed a thiamine-deficient diet have also shown a similar trunk-winding symptom (Hashimoto et al., 1970).

Like salmon and eels, the lamprey is a fatty species. The lipid content of lampreys ranges between 3 and 22.6%, depending on whether they are sampled from the sea or the river in the winter (Moore and Potter, 1976; Falandysz et al., 2000, 2001). According to Koli (1990), lampreys contain approximately 12%–15% lipids when they enter the spawning rivers. The lipid content of lampreys caught in rivers flowing into the Gulf of Bothnia between late September and mid-November 2016 was 15%–16% (Airaksinen et al., 2018; Kumar et al., 2022). As in salmon (Vuorinen et al., 2014b, 2020), the lipid content of lampreys reduces during pre-spawning fasting. In an investigation by Moore and Potter (1976), the lipid content of lampreys reduced from approximately 18% to 3% between October and March in both females and males, being similar in the muscle and whole body. For comparison, the lipid content of Baltic salmon ranged between 5 and 19%, depending on the feeding area and salmon age and size, during the feeding migration in the fall in nonsignificant M74 mortality years (Vuorinen et al., 2012; Keinänen et al., 2022), 3 and 13% during spawning migration to the northeastern rivers of the Gulf of Bothnia, and 1 and 5% four months later at spawning in the River Simojoki in 2004 (Vuorinen et al., 2020). However, in 1991–1993, when most salmon yolk-sac fry died of thiamine deficiency M74, the female muscle lipid content was 14% at spawning (Vuorinen et al., 2021). The lipid content of eel (*Anguilla anguilla*) caught in Finnish freshwaters was 5%–30% (Tulonen and Vuorinen, 1996).

The lipid-related thiamine deficiency M74 of salmon results from abundant feeding on fatty marine fish, especially young sprat (*Sprattus sprattus*) in the Baltic Proper (Mikkonen et al., 2011; Keinänen et al., 2012, 2018), but also from abundant feeding on herring (*Clupea harengus*) in the Bothnian Sea of the Gulf of Bothnia in years when there are large numbers of young—and fatty—herring (Vuorinen et al., 2020, 2021; Keinänen et al., 2022). Due to the high energy density of lipids (Kriketos et al., 2000) such a diet increases the need for thiamine (Woodward, 1994; Lonsdale and Marrs, 2019). In addition, and even more importantly, thiamine is depleted when acting as an antioxidant against lipid peroxidation of unsaturated fatty acids (Lukienko et al., 2000; Gibson and Zhang, 2002; Depeint et al., 2006), of which a polyunsaturated fatty acid of the *n-3* family (*n-3* PUFA), docosahexaenoic acid (DHA, 22:6*n-3*), is the most susceptible to lipid peroxidation (Tacon, 1996; Spector, 2000). In addition, DHA is the most common PUFA in Baltic herring and sprat (Keinänen et al., 2017), as well as in Baltic salmon (Keinänen et al., 2018). Because a diet containing fatty fish increases both the lipid and DHA content of fatty predatory fish species (Corraze and Kaushik, 1999; Keinänen et al., 2012, 2017, 2018; Futia et al., 2019), it also increases the susceptibility of their tissues to lipid peroxidation (Alvarez et al., 1998; Kjær et al., 2008; Keinänen et al., 2022). Thus, thiamine deficiency M74 has been shown to be associated with increased DHA content and lipid peroxidation in salmon tissues and eggs (Pickova et al., 1998, 2003; Lundström et al., 1999; Vuorinen et al., 2020).

Thiamine deficiency M74 has also caused mortality in yolk-sac fry of anadromous brown trout (*Salmo trutta* m. *trutta*) feeding in the Baltic Sea, but to a lesser degree than salmon (Amcoff et al., 1999; Landergren et al., 1999). As a less fatty species, brown trout are less prone to lipid peroxidation and lipid-related thiamine deficiency than salmon, in addition, their diet is more varied due to more benthic feeding habits (Landergren et al., 1999). The fattiest among the prey specimens of Baltic salmon such as the youngest sprat have also had the lowest thiamine concentrations, indicating a reduction of thiamine in their energy and fatty acid metabolism (Vuorinen et al., 2002; Keinänen et al., 2012, 2017). Hence, the fattiest fish species and specimens appear to be the most susceptible to lipid-related thiamine deficiency (Futia et al., 2019; Vuorinen et al., 2021), and especially when they fast before spawning as is the case with salmon (Vuorinen et al., 2014b) and lampreys (Savina and Gamper, 1998).

In the Baltic Sea, both lampreys and salmon feed on the clupeids herring and sprat (Koli, 1990; Hansson et al., 2001). Sprat is generally a fattier species, and its lipid percentage and consequently energy density varies more (Vuorinen et al., 2002; Keinänen et al., 2012; Røjbek et al., 2014). The lipid content has been highest especially in sprat in the very youngest specimens, but the lipid content has also differed between the areas: in both species it was higher in the Gulf of Bothnia than in the Baltic Proper from the fall of 2003 to the spring of 2004 (Vuorinen et al., 2012; Keinänen et al., 2017). The concentrations of DHA and *n-3* PUFAs were the highest in the youngest herring and sprat, in herring especially in the Gulf of Bothnia (Keinänen et al., 2017, 2022).

Lampreys eat fish by clinging to them and gnawing their skin and muscle (Renaud et al., 2009). The sucking tracts of lampreys have been detected at least on herring, sprat, smelt (*Osmerus eperlanus*), vendace (*Coregonus albula*), and salmon (Koli, 1990; Axén and Koski, 2017), all of which are fatty species (Kostamo et al., 2000; Käkelä et al., 2002); see Keinänen et al. (2012). Lampreys also eat benthic animals. At the start of their spawning migration in August, lampreys stop feeding, and their intestine, including the biliary system, atrophies (Savina and Gamper, 1998; Gamper and Savina, 2000; Konovalova et al., 2012). They are therefore not fed in the hatchery from the fall until the following spring, when they are stripped of their eggs in May.

Due to fasting, the nutrient and energy stores of the lamprey body must enable the development of the eggs and sperm and sustain physical activity and various physiological functions for 7–9 months until spawning, after which they die (Aronsuu, 2015). In exogenous vitellogenesis, pituitary gonadotropin stimulates ovarian follicle granulosa cells to secrete the steroid hormone 17β-estradiol, which in turn induces liver cells to synthesize vitellogenin and vitamin-binding proteins (Mommsen and Walsh, 1988). Extrahepatic lipid mobilization is induced by 17β-estradiol, which subsequently stimulates hepatic lipogenesis. Vitellogenin is a large phospholipoglycoprotein, which, in addition to lipids and proteins, transports ions, micronutrients and various compounds needed in embryonic development, to

Table 1
Timetable and description of river lamprey (*Lampetra fluviatilis*) handling, samplings, and determinations in 2014–2015. N = number of individuals, F/M = number of females/males.

Date	Action	Group	N (F/M)	Description	Sample	Determinations
September 2014	Catching and keep netting					
October 2014	Transfer to hatchery			5,600 lampreys arrived and were randomly distributed into several basins		
March 8, 2015	Sampling	LIV	20 (12/8)	Normally behaving	Liver	Total mass and length, body lipid content, liver thiamine
		SYMPT	18 (8/10)	Showing various symptoms		
		MORIB	20 (17/3)	Soon dying		
May 21–22, 2015	Stripping and sampling	Small	29 (29/–)	Classified first subjectively into “small” and “large” and then divided in half based on fatness index	Eggs, liver	Total mass and length, egg and liver thiamine
		Large	29 (29/–)			

the oocytes (Mommsen and Walsh, 1988). Thiamine is probably transported by vitellogenin or some other specific binding protein, as in chicken (Miller et al., 1981), although the exact role of each is thus far not known for fish. Because lamprey lipids are greatly reduced during pre-spawning fasting (Moore and Potter, 1976), the thiamine content is presumably reduced by acting as an antioxidant against lipid peroxidation (Gibson and Zhang, 2002) in addition to being transported to the growing oocytes (Miller et al., 1981; Mommsen and Walsh, 1988).

The symptoms typical of M74 in lampreys were thought to be related to thiamine deficiency, because they, like salmon, have a high lipid content, eat fatty marine prey fish, and have a long pre-spawning fasting period. This is especially because the lampreys that showed these symptoms preceding death were the largest and therefore supposedly fattiest specimens. It is known that the lipid content, and at the same time the average concentration of *n-3* PUFAs, particularly DHA, of salmonine feeding on fatty marine fish generally increases with age and growth (Corraze and Kaushik, 1999; Vuorinen et al., 2012). As the total thiamine (TotTh) concentration of the liver has appeared a sensitive indicator of the thiamine status in salmon (Koski et al., 2001; Vuorinen et al., 2020) and to have a negative relationship with the body lipid content in feeding-migrating salmon (Keinänen et al., 2022), the thiamine concentration of lamprey liver was expected to have a similar relationship to their body lipid content.

By analyzing the relationship of the hepatic TotTh concentration with the size and fatness indices of female and male lampreys and with the concentrations of thiamine and its components in the ovulated unfertilized eggs, the aim of the present study was to investigate (1) the association of the uncoordinated swimming symptoms of lampreys during pre-spawning fasting with their possibly impaired thiamine status and evaluate (2) the possible role of lipid-related thiamine deficiency in the occurrences of massive death of adult lampreys kept for the artificial spawning in the hatchery and (3) the effects of fatness and the thiamine status of female lampreys on the thiamine status of the eggs. This study is the first to examine thiamine concentrations in river lamprey.

2. Material and methods

2.1. Lamprey sampling

Having been caught in the River Perhonjoki in the latter half of October 2014, 5600 river lampreys [*Lampetra fluviatilis* (L.)] were transported to Luke’s Keminmaa Hatchery. At the hatchery, the lampreys were held in covered glass fiber rearing tanks with through-flowing water from the River Kemijoki until May 21–22, 2015, when they were stripped of their ovulated eggs. The timetable with experimental setup and sampling is summarized in Table 1.

Deaths of larger lampreys began after they displayed thiamine deficiency-like behavior in early 2015. Thus, on March 8, lampreys in the rearing tanks were sampled so that they could be grouped into three groups (LIV, SYMPT, and MORIB) based on a visual observation of their behavior and the severity of thiamine deficiency-like symptoms (M74 symptoms). Of the 58 lampreys sampled, (1) 20 were normally behaving (LIV); (2) 18 had M74 symptoms (SYMPT); and (3) 20 were moribund, apparently likely to die soon (MORIB). The sampled lampreys were killed using an overdose of Na_2CO_3-neutralized MS-222, sealed individually in coded zip-lock polyethylene bags, immediately frozen in dry ice, and preserved at −20 °C. The next day, they were shipped to the laboratory for analysis. In the laboratory, the mass and total length (with the aid of a string) of the frozen lampreys were measured, and in addition to Fulton’s condition factor (CF), the mass/length ratio was calculated as an indicator of fatness (hereafter fatness index or FI). The sex of the fish was recorded, and the liver was immediately sampled and sealed in Eppendorf tubes for thiamine analysis. The rest of the body was used to determine the body lipid content. The lampreys were also sampled and studied for possible diseases.

After the larger specimens in the rearing tanks had died by the spawning time in May, unfertilized eggs were sampled from 58 lampreys from the remaining normally behaving lampreys in the context of stripping the eggs. The eggs were sealed in Eppendorf tubes to determine their thiamine component concentrations. After stripping of the eggs, the respective females were killed and sampled as in March—described above—to measure the mass, length, and liver thiamine concentration; the body lipid content was not determined, because the eggs had been stripped. The sampled lampreys were grouped according to the size (fatness index) into 29 smaller (“small”) and 29 larger (“large”) specimens.

2.2. Chemical analyses

Thiamine was analyzed in the liver in March and May and in ovulated unfertilized eggs in May using high-performance liquid chromatography (HPLC), as described in Vuorinen et al. (2021). The measured thiamine components (nmol g^{-1} wet weight) were comprised of the phosphorylated thiamine derivatives, thiamine pyrophosphate (TPP) and thiamine monophosphate (TMP), and unphosphorylated or free thiamine (THIAM), which were summed up as total thiamine (TotTh). Briefly, approximately 0.5 g of the liver or eggs was weighed and homogenized with 2.0 ml of 2% tricarboxylic acid and incubated at 100 °C for 10 min. The sample was centrifuged, and the supernatant was washed with ethyl acetate-hexane (3:2). To convert thiamine components into their corresponding thiochromes, an aliquot of 425 µl of supernatant was taken, and 75 µl of 0.1% potassium hexacyanoferrate in 1.2 M (1.5 M for standards) sodium hydroxide was added. The sample was filtered prior to the HPLC run. The standards were subjected to the same procedure as the tissue samples with the mentioned exception. A subsample of the laboratory control sample was processed and analyzed along with the samples for quality assurance.

The total body lipid content (hereafter lipid content or lipid, %) of females and males in March was determined in the whole body homogenate on the basis of the Schmid–Bondzynski–Ratzlaff procedure (ISO, 2004), as in Keinänen et al. (2017). Briefly, a sample was first digested with hydrochloric acid. After ethanol addition, lipid was extracted with diethyl ether and light petroleum. Finally, solvents were removed by evaporation and the precipitate was weighed.

2.3. Statistical calculations

Parameters were tested for normality (Kolmogorov–Smirnov test), and a Levene's test was used to test homogeneity among variances. Accordingly, parametric or non-parametric tests were used. For comparisons between the group mean values, a significance level of $\alpha = 0.05$ was used. Spearman rank correlations were applied to examine correlations between the parameters. A one-way ANOVA with a Student–Newman–Keuls (SNK) post-hoc test was applied to test the differences in the mean values of the various parameters (except for thiamine components, not normally distributed) between the groups of lampreys sampled in March (females and males separately for the groups LIV, SYMPT, and MORIB) or in May (only females, the groups "small" and "large") (Table 1). For differences between the group means in the thiamine component concentrations of samples in March and May, a Wilcoxon/ Kruskal–Wallis test with the Dwass, Steel, Critchlow–Fligner (DSCF) multiple comparison post-hoc analysis was applied. The differences in the mean values of various parameters between the sexes were tested by the t-test.

A principal component analysis (PCA) was carried out for the multivariate statistical comparisons of detailed parameter effects on lamprey groups in March with the sexes separately (Kvalheim and Karstang, 1987). Prior to this, the parameter data was log-transformed and subsequently standardized (parameter deviations homogenized) to prevent the abundant components with large variances from dominating the analysis. In the PCA, the samples originally positioned in a multidimensional space (as many coordinates as parameters determined) were plotted in a newly formed two-coordinate system of principal components, PC1 and PC2. PC1 accounted for as much of the original data variability as possible and PC2 explained as much as possible of the remaining variation uncorrelated with the variation already explained by PC1. As a result, a biplot graph with the principal components PC1 and PC2 was created to demonstrate (dis)similarities between the lamprey groups and to indicate correlations between the parameters. Soft independent modeling of class analogy (SIMCA) was used to quantify the differences at the level of $\alpha = 0.05$ between the groups' pairs (Wold and Sjöström, 1977).

The statistical calculations were performed using the Statistical Analysis System software (SAS ver. 9.4), apart from PCA and SIMCA, which were carried out using Sirius software (ver. 8.5, Pattern Recognition Systems, Norway, www.prs.no). The figures were drawn with Origin Pro 2022 (OriginLab Co., Northampton, Massachusetts, USA).

3. Results

Since early February 2015, lampreys, especially the largest specimens—by visual observation—had increasingly been dying in the hatchery. Overall, 27% of the lampreys died. Before death, they showed uncoordinated swimming, and at death, they were often coiled. Of the 58 lamprey specimens sampled on March 8, in the MORIB group, 85% were females, while in the SYMPT group, 44%, and in the LIV group, 60%, were females. Hence, the lampreys that died after showing typical thiamine deficiency symptoms were mostly females.

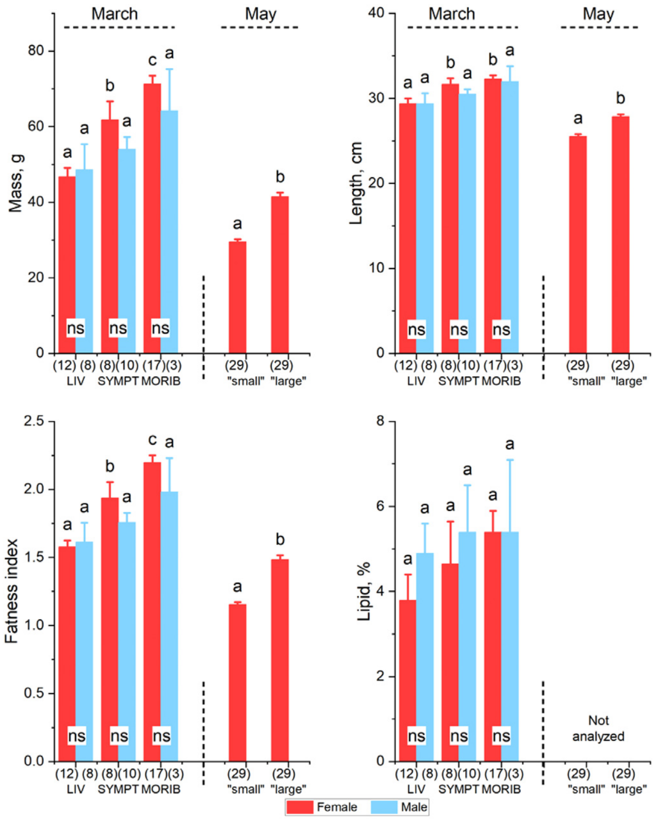

Fig. 1. Mass, length, fatness index, and lipid content of river lamprey (*Lampetra fluviatilis*) females and males in March (LIV = normal; SYMPT = showing abnormal behavior leading to death; MORIB = close to death) and of females in May at the stripping of the eggs ("large" and "small"). A different letter indicates a significant ($p < 0.05$) difference between the groups among females or males. The significance (ns = $p > 0.05$) of difference between the females and males within groups in March is also indicated. The number of observations is given in parentheses.

The mass and fatness index of females differed significantly between all groups and was largest in the MORIB group and smallest in the LIV group (Fig. 1), and the CF of females was also significantly the largest in the MORIB group (Suppl. Table 1). Females in the SYMPT and MORIB groups were also significantly ($p < 0.05$) longer than the LIV females (Fig. 1). The fatness index was significantly and positively (Tables 2 and 3) correlated with the body lipid content and was assumed to be illustrative of the lipid content of the lampreys, which the CF did not, as Jolley et al. (2015) also observed.

The similar differences in the CF and fatness index, as well as the mass and length of males between the groups, were not significant due to their small number (3) in the MORIB group and large variation (Fig. 1 and Suppl. Table 1). Nor were the differences significant ($p>0.05$) in these parameters between the females and males within the three groups in March. However, the lipid content tended to be larger in the SYMPT and MORIB groups than in the LIV group in both females and males (Fig. 1 and Suppl. Table 1).

The differences in all these parameters between the groups were not always statistically significant due to individual variation within the groups, because the sampling into groups was

Table 2
Spearman correlations (with the significance below and significant values in boldface) between the parameters [mass (g), length (cm), fatness index (FI), Fulton's condition factor (CF), body lipid content (% wet weight), total thiamine concentration (TotTh, nmol g^{-1} wet weight) in the liver, and hepatic TotTh/body lipid] of river lamprey (*Lampetra fluviatilis*) females in March 2015 (N = 37).

	Length	FI	CF	Lipid	TotTh	TotTh/Lipid
Mass	**0.884**	**0.985**	**0.521**	**0.551**	−0.238	**−0.487**
	<.0001	<.0001	0.001	0.000	0.156	0.002
Length		**0.810**	0.135	**0.535**	−0.132	**−0.420**
		<.0001	0.426	0.001	0.438	0.010
FI			**0.620**	**0.527**	−0.275	**−0.488**
			<.0001	0.001	0.099	0.002
CF				0.169	**−0.388**	−0.292
				0.316	0.018	0.079
Lipid					**−0.421**	**−0.906**
					0.009	<.0001
TotTh						**0.729**
						<.0001

Table 3
Spearman correlations (with the significance below) between the parameters of river lamprey (*Lampetra fluviatilis*) males sampled in March 2015 (N = 21). See the explanations in Table 2.

	Length	FI	CF	Lipid	TotTh	TotTh/Lipid
Mass	**0.975**	**0.988**	0.106	**0.583**	**−0.552**	**−0.661**
	<.0001	<.0001	0.646	0.006	0.010	0.001
Length		**0.942**	−0.017	**0.613**	**−0.509**	**−0.678**
		<.0001	0.942	0.003	0.018	0.001
FI			0.213	**0.576**	**−0.555**	**−0.648**
			0.354	0.006	0.009	0.002
CF				−0.039	0.023	0.027
				0.867	0.920	0.907
Lipid					**−0.567**	**−0.963**
					0.007	<.0001
TotTh						**0.688**
						0.001

based on the visual observation of lamprey behavior (Suppl. Table 1). However, in all these parameters, the tendency was that the values were smallest in the LIV group and largest in the MORIB group. This was especially evident in females. Thus, the lipid content was significantly and positively correlated with the mass and length, in addition to the fatness index, of both lamprey females and males (Tables 2 and 3).

In all three groups, the hepatic TotTh concentration was significantly smaller in females than in males (Fig. 2). In both, it tended to be smallest in the MORIB group and largest in the LIV group (Fig. 2 and Suppl. Table 2). In males, the hepatic TotTh concentration was significantly and negatively correlated with the body mass and length, and with the fatness index (Table 3). For females, a negative correlation was significant only with CF (Table 2), apparently because the largest and fattiest dead fish with the lowest hepatic TotTh concentration were mostly females. In both sexes, the hepatic TotTh concentration was significantly and negatively correlated with the lipid content (Tables 2 and 3 and Fig. 3). The coefficient of determination in these models was rather low but significant with a clear trend and lower in females than in males, apparently because females with the lowest thiamine concentrations had died before sampling. The hepatic TotTh to lipid ratio was significantly and negatively correlated with the mass, length, and fatness index in females and males (Tables 2 and 3).

According to the biplots, the principal components 1 and 2 (PC1 and PC2) in PCA separated the females of the LIV and MORIB group (Fig. 4) with a slight overlap, although the difference was not significant ($p > 0.05$, SIMCA). In females, PC1 and PC2 explained 80% of the variation with the parameters mass, body lipid, and hepatic TotTh contributing most to PC1, while length contributed to PC2. Mass and lipid had a strong positive correlation, while hepatic TotTh was strongly negatively correlated with the two. In females, hepatic TotTh was more associated

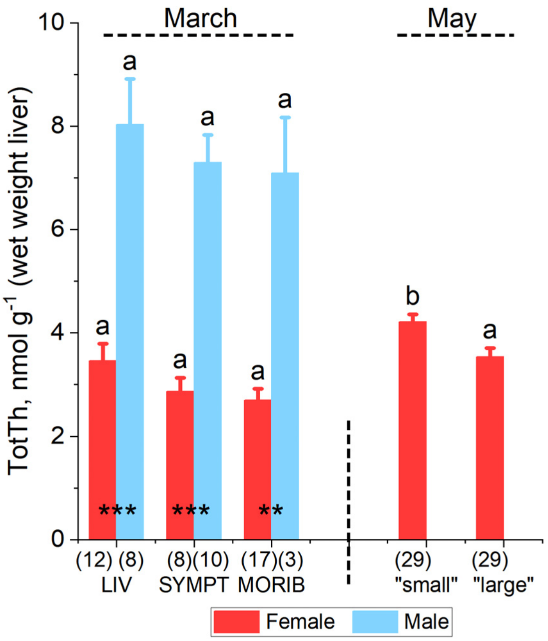

Fig. 2. The mean (± SE) hepatic total thiamine (TotTh) concentration of river lampreys (*Lampetra fluviatilis*) in March (LIV = normal, SYMPT = showing abnormal behavior leading to death, and MORIB = close to death) and at spawning in May ("small" and "large"). A different letter indicates a significant ($p < 0.05$) difference between the means among groups in females or males. A significant (** = $p < 0.01$ and *** = $p < 0.001$) difference between the females and males within the groups in March is also indicated. The number of observations is given in parentheses.

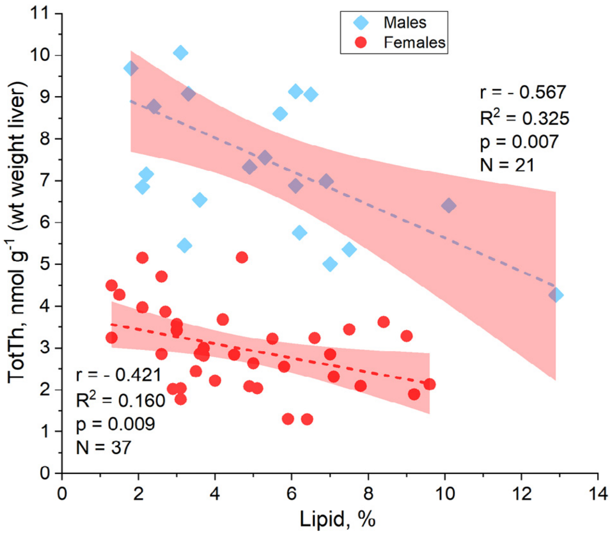

Fig. 3. Relationships of the hepatic total thiamine (TotTh) concentration with the body lipid content of river lamprey (*Lampetra fluviatilis*) females and males in March. Spearman correlation coefficients (r), coefficient of determination (R^2), significance (p), and the number of observations (N) are given.

Table 4
Significant ($p < 0.05$) difference indicated by a dissimilar letter between the mean values of the groups of river lamprey (*Lampetra fluviatilis*) females in total body mass, total body length, fatness index (FI) and liver total thiamine concentration (TotTh, nmol g^{-1}wet weight) in March and at spawning in May.

Sampling	Group	Mass	Length	FI	TotTh
March	LIV	b	c	b	abc
March	SYMPT	c	d	c	ab
March	MORIB	d	d	d	a
May	“small”	a	a	a	c
May	“large”	b	b	b	b

with the LIV than the MORIB group, and body lipid and mass were associated with the MORIB group. In males, the groups were intermingled with a slight shift ($p > 0.05$, SIMCA) and PC1 and PC2 together explained 88% of the variation (Fig. 4). PC1 was mostly influenced by mass, length and hepatic TotTh, and body lipid contributed to PC2. In males, mass was more associated with the MORIB group than with the LIV group, and mass and body lipid negatively correlated with hepatic TotTh. Thus, in both females and males, the hepatic TotTh concentration was strongly and negatively correlated with the body lipid content and body mass.

Among lamprey females sampled in May at the stripping of the eggs, the fatness index was significantly higher in the “large” group than in the “small” group, as the body mass and length were (Fig. 1). On the contrary, the hepatic TotTh concentration was significantly lower in the “large” group than in the “small” group (Fig. 2).

The mass, length, and fatness index of lamprey females in the “small” and “large” groups sampled in May were significantly ($p < 0.05$) smaller than those of females in the SYMPT and MORIB groups sampled in March (Table 4 and Fig. 1). The hepatic TotTh concentration of females in the “small” group was significantly ($p < 0.05$) larger than in the “large”, SYMPT, and MORIB groups, and tended to be larger than in the LIV group (Table 4 and Fig. 2). Including all females, i.e., those sampled in March and May, the hepatic TotTh concentration was significantly and negatively correlated with the fatness index (Fig. 5).

The THIAM concentration of the ovulated unfertilized eggs was significantly higher in the “small” group than in the “large” group, as was the TotTh concentration (Table 5). The THIAM concentration of the eggs, with the two groups combined, was

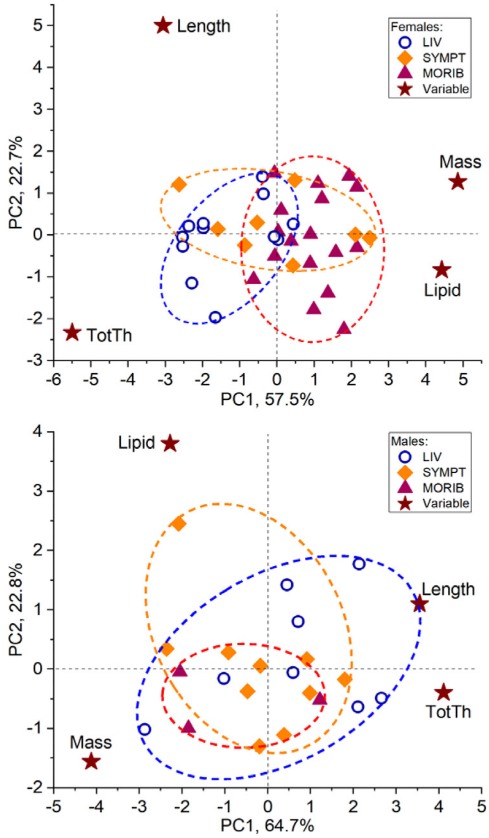

Fig. 4. Biplots from the PCA model with principal components (PC1 and PC2) for river lamprey (*Lampetra fluviatilis*) females (above) and males (below) of the three groups: LIV (normal), SYMPT (showing abnormal behavior leading to death), and MORIB (close to death) in March two months before spawning. Parameters: hepatic total thiamine concentration (TotTh, nmol g^{-1} wet weight), body lipid content (% wet weight), body mass (g), and length (cm). According to the SIMCA test, none of the group pairs in females or males were separated significantly ($p > 0.05$).

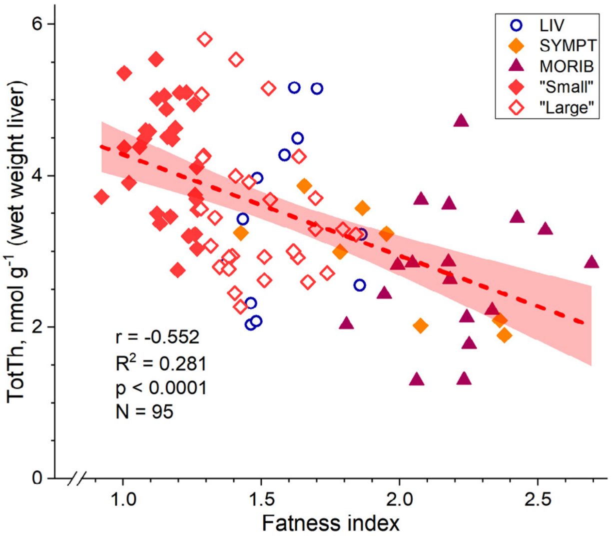

Fig. 5. Relationships of the hepatic total thiamine (TotTh) concentration with the fatness index (mass per length) of river lamprey (*Lampetra fluviatilis*) females in March and May. Spearman correlation coefficient (r), coefficient of determination (R^2), significance (p), and the number of observations (N) are given.

Table 5
The mean (±SE, with the ranges below in parentheses) concentrations (nmol g^{-1}wet weight) of thiamine components (TPP = thiamine pyrophosphate, TMP = thiamine monophosphate, and THIAM = free or unphosphorylated thiamine) and total thiamine (TotTh) in ovulated unfertilized eggs of smaller ("small", N = 29) and larger ("large", N = 29) river lampreys (*Lampetra fluviatilis*) at spawning in May. A different superscript letter indicates a significant ($p < 0.05$) difference in parameter means between groups. The number of observations was 29 in both groups.

	"small"			"large"		
Unfertilized eggs						
TPP, nmol g^{-1}	5.039	±	0.186^a	4.580	±	0.191^a
	(2.800	–	7.187)	(1.497	–	6.764)
TMP, nmol g^{-1}	1.280	±	0.049^a	1.141	±	0.051^a
	(0.520	–	1.964)	(0.393	–	1.755)
THIAM, nmol g^{-1}	0.348	±	0.021^b	0.245	±	0.017^a
	(0.110	–	0.596)	(0.068	–	0.474)
TotTh, nmol g^{-1}	6.667	±	0.241^b	5.966	±	0.250^a
	(3.584	–	9.649)	(1.958	–	8.992)

Table 6
Spearman correlations of the concentrations (nmol g^{-1} wet weight) of thiamine components (TPP = thiamine pyrophosphate, TMP = thiamine monophosphate, and THIAM = free, unphosphorylated thiamine), and total thiamine (TotTh) of the ovulated unfertilized eggs with the body mass (g), fatness index (mass per length) and hepatic TotTh concentration of river lamprey (*Lampetra fluviatilis*) females at the stripping of the eggs in May. The significance of the correlations is also given below the correlation coefficient, of which significant ones are emphasized in bold face. There were 58 observations.

	Mass	Fatness index	Hepatic TotTh
Unfertilized eggs			
TPP	−0.137	−0.216	**0.365**
	0.305	0.103	0.005
TMP	−0.207	**−0.303**	**0.445**
	0.120	0.021	0.001
THIAM	**−0.332**	**−0.408**	**0.539**
	0.011	0.002	<.0001
TotTh	−0.198	**−0.281**	**0.422**
	0.136	0.033	0.001

positively and highly significantly correlated with the hepatic TotTh concentration (Fig. 6). The concentrations of the phosphorylated thiamine components and TotTh in the eggs were likewise significantly and positively correlated with the hepatic TotTh concentration so that the correlation, after THIAM, was the second strongest for TMP, then for TotTh, and the weakest for TPP (Table 6).

The THIAM concentration in the eggs was significantly and negatively correlated with the fatness index and body mass (Table 6 and Fig. 6). The negative correlation with the fatness index was also significant for the concentrations of egg TMP and TotTh, but not for TPP (Table 6).

4. Discussion

During the pre-spawning fast, the thiamine status of the lampreys, inferred from the hepatic TotTh concentration, was the poorer the fattier they were. Since the largest lampreys were the fattiest, they were the first to develop M74-like symptoms and die from thiamine deficiency. For females, however, the thiamine status was significantly poorer than for the males, apparently because in females both lipids and thiamine had been consumed not only for energy metabolism but also for growing oocytes. Therefore, most of the dying lampreys in March, i.e. towards the end of the pre-spawning fast that had started in early autumn, were large females. The smallest of the females survived until spawning in May, but even among them, the thiamine status was poorest in the largest, fattiest females. The poor thiamine status of the lamprey females led to low concentrations of all thiamine components in eggs, although the association was strongest with the THIAM concentration. Likewise, a low THIAM concentration in the eggs had the clearest association with large female fatness index and mass.

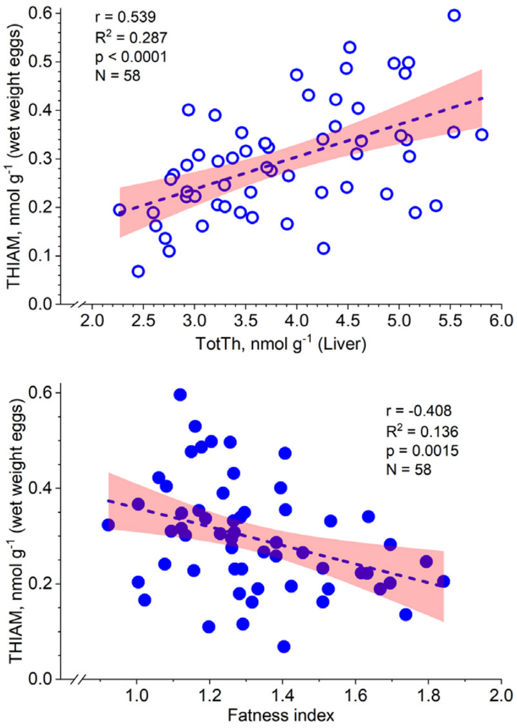

Fig. 6. Relationship of the free thiamine (THIAM) concentration in the ovulated unfertilized eggs with the hepatic total thiamine (TotTh) concentration (above) and the fatness index (mass per length, below) of river lamprey (*Lampetra fluviatilis*) females at the stripping of the eggs in May. Spearman correlation coefficient (r), coefficient of determination (R^2), significance (p), and the number of observations (N) are given.

4.1. The fattiest females with the poorest thiamine status were the first to die of thiamine deficiency

A small hepatic TotTh concentration of pre-spawning lampreys, both females and males, was associated with high lipid content similarly to feeding and spawning salmon (Vuorinen et al., 2020; Keinänen et al., 2022). Accordingly, in PCA, hepatic TotTh was inversely correlated with lipid in both sexes. The highest lipid percentages of lampreys in March were close to the average percentages of spawning salmon in the years when the M74 syndrome was at its worst (around 10% in 1991–1993), but higher than in spawning salmon in the years with a nonsignificant incidence of M74 (around 5%) (Vuorinen et al., 2020, 2021).

The symptoms of lampreys, such as uncoordinated swimming before the death, resembled those of thiamine deficiency, which has thus far been documented in adults and yolk-sac fry of Baltic salmon and brown trout suffering from M74 (Bylund and Lerche, 1995; Amcoff et al., 1999; Landergren et al., 1999; Keinänen et al., 2000), as well as in other salmonine species in North America (Thiamine Deficiency Complex, TDC) (Fisher et al., 1995; Futia et al., 2017), and experimentally in eel (Hashimoto et al., 1970). Apart from being fattier, the symptomatic and moribund lampreys were larger than the lampreys of the LIV group, and the moribund females also had a larger CF. Similarly, during the 2002–2003 reproductive period in the hatchery of the Korpela hydroelectric powerplant, the largest lampreys behaved abnormally, and their mortality was increased.

Because the symptoms of thiamine deficiency and mortalities were found in the largest lampreys as early as at the beginning of 2015, the largest females with the lowest hepatic TotTh concentration may have already died before the March sampling. This assumption is supported by the fact that at sampling in March the symptomatic and moribund lampreys were larger and fattier than the asymptomatic specimens, and the symptomatic and moribund females tended in general to be larger and fattier than the males in the respective groups (the SYMPT and MORIB groups). Although the size indices were not good predictors of hepatic TotTh in March, the negative relationship between the hepatic TotTh concentration and lipid content was also clear for females. Similarly, in the years with a high M74 incidence in the 1990s, the largest salmon females were usually those whose offspring suffered from thiamine deficiency (M74 females) (Mikkonen et al., 2011; Vuorinen et al., 2021). Furthermore, the mean CF of salmon M74 females was higher in most years than that of non-M74 females (Vuorinen et al., 2021), and a high mean annual CF of pre-spawning and spawning salmon predicted higher M74 mortality (Mikkonen et al., 2011).

During pre-spawning fasting, thiamine consumption was apparently higher in females that had mobilized their lipid stores for oocyte development in addition to basal metabolism and physical activity (Tocher, 2003). This was indicated by significantly smaller hepatic TotTh concentrations in females, which were 38%–43% of the concentrations in males. The lowest hepatic TotTh concentrations in female MORIB lampreys (1.3–4.7 nmol g^{-1}) were near the lowest concentrations in spawning salmon in 1995, a severe M74 year (1.2–2.2 nmol g^{-1}) (Koski et al., 2001), although these analyses were performed using different methods. In turn, in female LIV lampreys, the hepatic TotTh concentrations (2.0–5.2 nmol g^{-1}) were roughly half those in spawning salmon in 2004, a year with a nonsignificant M74 incidence (6.3–10.4 nmol g^{-1}) (Vuorinen et al., 2020).

4.2. Still at spawning, thiamine status was poorest in the largest and fattiest females

At spawning in May, the hepatic TotTh concentration was higher in the smaller lamprey females ("small" group), and their fatness index was lower than in the larger ones ("large" group). However, in "small" females the hepatic TotTh concentration (2.8–5.5 nmol g^{-1}) was also somewhat higher, and the fatness index significantly lower than in LIV lampreys sampled in March. These facts suggest that the fattiest females with the lowest hepatic TotTh concentrations had also died after the March sampling by spawning in May. The females' smaller size in both May groups than of the females in the LIV group consistently suggests that only the smallest and thus the leanest females had survived until spawning. In the final stages of the oocyte development shortly before spawning, the mass and length of lampreys can decrease by as much as 10% (Ojutkangas and Valtonen, 1998; Dziewulska and Domagala, 2009), which may have hampered comparisons of the lampreys sampled in March and May. However, even when considering the mass of stripped eggs (circa 5 g per female), the mass of the lamprey females at stripping in May was approximately 14% and the length 9% smaller than in the LIV group. All these comparisons give a further basis to the hypothesis that the largest and at the same time the fattiest females, with the poorest thiamine status, died after the March sampling before spawning.

4.3. Lamprey mortality increased with the abundance of fatty prey fish

The feeding areas of the River Perhonjoki lampreys are probably in the southern Bothnian Bay and northern Bothnian Sea of the Gulf of Bothnia, but the extent of the feeding area and migration in the sea are unknown. However, it is known that lampreys feed on the same fatty pelagic fish species as salmon (Koli, 1990), and lampreys feed on salmon (Axén and Koski, 2017). Herring has been the dominant pelagic prey fish species in the Gulf of Bothnia (Hansson et al., 2001; Salminen et al., 2001; Mikkonen et al., 2011; Jacobson et al., 2018). The herring 2014 year class was strong, the largest since the exceptionally large herring year-class of 2002 in the Gulf of Bothnia (ICES, 2021a). Consequently, among the 2nd sea-year salmon females that returned to spawn in the River Simojoki in the Gulf of Bothnia in 2015–2017, after four non-M74 years, there were M74 females, which according to the fatty acid signature analysis (FASA) had fed on herring in the Gulf of Bothnia (Keinänen et al., 2017; Vuorinen et al. unpubl.). In 2016, the proportion of these M74 females which had the opportunity to eat abundant herring year-class for two years, was the highest (Vuorinen et al. unpubl.). Apparently, Gulf of Bothnia herring were also fatty then because their CF was even higher in 2014 than in 2013 and 2015, although the herring were not lean in those years either (https://www.luke.fi/uutinen/suomenlahdella-silakka-laihaa-selkamerella-lihavaa/ and Jari Raitaniemi, pers. comm.). The strong herring year class was possibly also reflected in the higher mean body mass of older herring age groups (>3 years) due to cannibalism than in the preceding and the following year classes (ICES, 2021a).

The studied lampreys had probably been feeding on those older fatty herring and young herring that were abundant during the summer of 2014, i.e., just before the spawning migration. Some sprat also migrate from their spawning areas of the Baltic Proper to the southern Gulf of Bothnia, where they do not reproduce (Aro, 1989). According to the annual fish survey of the fall of 2014, there were somewhat more sprat in the Gulf of Bothnia than in most years, and they were large and in good condition (Pönni, 2015, 2021). The sprat 2014 year class of the Baltic Sea was the third largest in the entire 1974–2021 survey period (ICES, 2021a; Pönni, 2022), and according to FASA, some of the salmon M74 females in 2015–2017 had preyed on sprat in the Baltic Proper (Vuorinen et al. unpubl.). Furthermore, a record number of salmon migrated through the Gulf of Bothnia to spawn in the Bay of Bothnia rivers in 2014 (Pakarinen et al., 2020; ICES, 2021b) and lampreys had an opportunity to feed on them (Axén and Koski, 2017). These species and smelt are loose-scaled or "scaleless", providing skin that is easy to penetrate with lamprey teeth (Renaud et al., 2009). Lampreys, like predator fish in general, presumably feed on the prey that is most readily available and of a suitable size and consistency.

Interestingly, there was exceptionally high mortality of lampreys in the hatchery of the Korpela hydroelectric power plant in the winter of 2002–2003. The mortality rate was about 11%, while it was usually a few percent, and the lampreys behaved strangely, sinking to the bottom of the rearing tanks. No cause of mortality was found at that time, and no water quality or technical cause of mortality could be demonstrated. However, in the Gulf of Bothnia the herring 2002 year class was exceptionally strong (ICES, 2021a; Pönni, 2021). In 2004, among the 32 2nd sea-year salmon female ascendants that were included in M74 monitoring, a single salmon M74 female in monitoring with a yolk-sac fry mortality of 100% had been eating herring in the Gulf of Bothnia (Keinänen et al., 2018, 2022; Vuorinen et al., 2020). Instead, according to the FASA, none of those that had been eating sprat in the Baltic Proper suffered from M74 (Keinänen et al., 2018).

4.4. Poor thiamine status indicates fish-based marine diet rich in *n-3* PUFAs

Feeding on fatty marine prey fish in general increases both lipid content and the concentration of *n-3* PUFAs, and especially that of DHA, in fatty fish (Alvarez et al., 1998; Corraze and Kaushik, 1999; Keinänen et al., 2017). In 2003–2004, the prey fish of salmon, i.e., smallish herring and sprat, were fattier in the Bothnian Sea than in the Baltic Proper (Vuorinen et al., 2012; Keinänen et al., 2017). The proportions of DHA and *n-3* PUFAs were therefore also higher in Bothnian Sea salmon than in salmon caught in the Baltic Proper (Keinänen et al., 2018), and also because the proportions of these fatty acids were higher in herring than in sprat (Røjbek et al., 2014; Keinänen et al., 2017). In addition, in young herring, as in young sprat, the concentrations of these fatty acids are higher than in older specimens (Keinänen et al., 2017). The very large herring 2002 year class therefore increased the supply of these fatty acids for lampreys that ascended in 2002 after their last or only sea year, as it did for salmon that ascended in 2004 after their two feeding years (2002–2003). Similarly, after the recruitment of a strong herring year class in the Gulf of Bothnia in 2014, there was a large number of young and fatty herring (ICES, 2021a), which provided predators abundantly with DHA and *n-3* PUFAs (Keinänen et al., 2017, 2022).

The lipid content of lampreys ascending rivers in the fall (Koli, 1990; Airaksinen et al., 2018; Kumar et al., 2022) was more than twice that of ascending salmon in 2004 and 2016 (Vuorinen et al., 2020; Vuorinen et al. unpubl.). Apparently, the concentration of DHA in lampreys was also higher than in those salmon. In contrast, in eels, which as catadromous species eat freshwater fish during the growth period, the proportions of saturated and monounsaturated fatty acids and *n-6* PUFAs, which are more typical of freshwaters, accumulated in high proportions, whereas the proportion of DHA remained small (Gómez-Limia et al., 2021).

DHA, which is the most abundant PUFA in the prey fish eaten by both lampreys and salmon, is the most susceptible fatty acid to lipid peroxidation (Tacon, 1996; Spector, 2000). As thiamine is consumed when acting as an antioxidant against lipid peroxidation (Manzetti et al., 2014; Vuorinen et al., 2020), the smallest hepatic TotTh concentrations in the fattiest lampreys apparently resulted, similarly to the fattiest salmon (Keinänen et al., 2022), from the depletion of thiamine in lipid peroxidation. Thus, the cause of the death of the lampreys that died in February–March, and still after that before spawning was their poor thiamine status, and most of the dead lampreys were the largest and fattiest females.

4.5. Body lipids and thiamine are consumed in energy metabolism and transferred to oocytes

Estimated from the lipid content of all lampreys sampled in March in the present study and those that entered rivers for spawning in different falls (Koli, 1990; Airaksinen et al., 2018; Kumar et al., 2022), the lipid content of the River Perhonjoki lampreys would have decreased by at least 55%–76% by March. In the River Severn, the lipid content of lampreys decreased by 83% between October and March (Moore and Potter, 1976). According to Corraze and Kaushik (1999), muscle lipids are reduced in salmonines by 40%–60%, and visceral lipids by more than 70%, before spawning. In Baltic salmon, muscle lipids were reduced by 50% during the whole spawning run from the southern Baltic Sea to the River Simojoki in the northeastern Gulf of Bothnia and approximately four months of fasting, i.e., from the spring to the fall in 2004 (Vuorinen et al., 2020). In lampreys, which fast for a total of nine months after entering the river before spawning, the body lipid content will probably decrease during the pre-spawning fast even more than in salmon, although the energy consumption is supposedly smaller during the winter in cold water.

A major role of fish lipids is to store and provide metabolic energy, and lipids are also used for developing oocytes (Tocher, 2003). Fatty acids liberated from body lipids are catabolized in mitochondria via β-oxidation and further in the tricarboxylic acid cycle (TCA) for energy needs in the form of adenosine triphosphate (ATP) (Tocher, 2003). Because thiamine is vital in this mitochondrial oxidative metabolism, its availability determines whether and how much ATP is produced (Combs and McClung, 2017; Lonsdale and Marrs, 2019). In the fall and winter, the mitochondria in the hepatocytes of lampreys undergo reversible metabolic suppression, and their ATP production decreases (Gamper et al., 2001; Emel'yanova et al., 2007), which may save the thiamine reserves, because some thiamine is consumed in TCA. Despite the deceleration in the metabolism, vitellogenin synthesis and exogenous vitellogenesis are in progress when the body nutrients are used for the developing oocytes (Agalakova et al., 2016). Vitellogenin synthesis is accelerated in lamprey females before spawning, when oxidative metabolism and hepatic lipolysis are activated in March–April (Brailovskaya et al., 2007; Emel'yanova et al., 2007). The massive lamprey deaths in the present study took place at these times.

The hepatic TotTh concentration of lamprey females at spawning was approximately only half that of salmon females at spawning in 2004 with a nonsignificant M74 incidence (Vuorinen et al., 2020) and close to those in salmon during a year of severe M74 (Koski et al., 2001). Because thiamine homeostasis and thiamine in triphosphate form are also essential for the proper functioning of the nervous system (Gibson and Zhang, 2002), and because thiamine is essential for the formation of ATP (Combs and McClung, 2017; Lonsdale and Marrs, 2019), thiamine-deficient lampreys had impaired energy production leading to the various symptoms and ultimately death. In addition, thiamine is transported to the oocytes during vitellogenesis in females (Miller et al., 1981), meaning they suffered from more serious thiamine deficiency than the males.

4.6. Low egg thiamine concentrations related to female fatness and poor thiamine status

In the eggs, the concentrations of all thiamine components were positively related to the hepatic TotTh concentration of the lamprey females, which indicates that the extent of the thiamine accumulated in the eggs depended on the females' thiamine status. Similarly, there was a correlation between the hepatic and egg TotTh concentration in salmon in the years of high M74 incidence (Koski et al., 2001). Additionally, in 2004, a year of non-significant M74 incidence, all ovarian thiamine components were positively correlated with the hepatic TotTh concentration among spawning migrating and spawning salmon (Vuorinen et al., 2020; Keinänen et al. unpubl.).

Of the thiamine components in lamprey eggs, the relationship with the hepatic TotTh concentration was strongest for the concentration of THIAM, an unbound reserve thiamine, the concentration of which in salmon eggs was strongly associated with the THIAM concentration of the muscle (Vuorinen et al., 2020). Overall, thiamine is largely located in the muscle tissue, which is the largest tissue in fish. In salmonine muscle, the THIAM concentration was smallest in the fattiest species and specimens with the highest DHA and *n-3* PUFA concentrations (Futia et al., 2019; Vuorinen et al., 2020; Keinänen et al., 2022). The negative correlation of the egg THIAM concentration with the fatness index and mass of the lamprey females indicated that, similarly to salmon (Vuorinen et al., 2020; Keinänen et al., 2022), the fattiest females had the least amount of THIAM to be transferred to the oocytes, because in them THIAM has been consumed the most as an antioxidant against lipid peroxidation (Vuorinen et al., 2020; Keinänen et al., 2022).

The concentration and proportion of THIAM of the thiamine components in lamprey eggs varied most, similarly to salmon eggs (Keinänen et al., 2014; Vuorinen et al., 2021). In the eggs of the "small" lampreys, the mean THIAM concentration (0.353 with a range 0.110–0.596 nmol g^{-1}) was higher than in the "large", fattier lampreys (0.245, 0.068–0.474 nmol g^{-1}), indicating a higher thiamine consumption in the fattier lampreys during pre-spawning fasting. These lowest concentrations were near the lowest THIAM concentrations recorded in the eggs throughout the Finnish M74 monitoring of the salmon of the Bothnian Bay rivers since 1994 (range 0.060–13.040 nmol g^{-1}) (Vuorinen et al., 2021).

The difference in the TotTh concentration between lamprey and salmon eggs is due to a considerably higher TPP concentration in the lamprey eggs. The TPP concentration in lamprey eggs (1.50–7.19 nmol g^{-1}) was 4–9 times higher than in salmon eggs (0.38–0.83 nmol g^{-1}) at similar TotTh concentrations. Throughout the M74 monitoring, the TPP concentration was also lower [0.01–3.34 nmol g^{-1} (Vuorinen et al., 2021)] in the eggs of salmon from the Bothnian Bay rivers than in lamprey eggs. TPP appear to be transported during vitellogenesis (Miller et al., 1981; Mommsen and Walsh, 1988) to the developing oocytes in larger proportions in lampreys than in salmon, probably due to a species-specific characteristic. However, the fatness index or size of the lampreys did not correlate with the egg TPP concentration. Similarly, in salmon muscle the TPP concentration did not correlate with the lipid content during spawning (Vuorinen et al., 2020), although there was a positive correlation between these parameters in salmon during the feeding migration (Keinänen et al., 2022).

The TPP varied least among the thiamine components in the eggs of lamprey, similarly to salmon (Vuorinen et al., 2021), apparently because TPP is the biologically active form of thiamine, and >90% of it is bound to the enzymes in the cytosol and in the mitochondria (Depeint et al., 2006; Combs and McClung, 2017). TPP in general accounts for a major part of thiamine in vertebrate soft tissues such as the muscle, brain, and liver (Combs and McClung, 2017). In salmonine muscle, as well as in sprat and herring, the TPP proportion was higher in fattier than in leaner specimens and species (Futia et al., 2019; Keinänen et al., 2022), and TPP accounted for 80%–88% of the muscle TotTH in Baltic salmon on their spawning run and at spawning (Vuorinen et al., 2020).

Unlike salmon eggs, the concentration of TMP in lamprey eggs was larger than the THIAM concentration. In salmon eggs, the TMP concentration was always lower than that of THIAM at similar TotTh concentrations to lamprey eggs (Vuorinen et al., 2021). The TMP concentration is linked with the THIAM concentration both in lampreys and salmon, probably because the phosphorylation of THIAM to TMP is a critical step in the transport of thiamine across the membranes (Depeint et al., 2006; Manzetti et al., 2014). In lampreys, the TMP concentration was larger than in salmon, apparently because their TPP concentration was high, and TPP can be hydrolyzed into TMP (Combs and McClung, 2017).

4.7. Low egg free thiamine concentration most clearly associated with low thiamine status

Overall, the thiamine status of lampreys, as in salmon, was best reflected in the THIAM concentration of the thiamine components of the eggs. The M74 and TDC symptoms and mortality of yolk-sac fry in salmonines are specifically related to a low THIAM concentration of the ovulated unfertilized eggs (Keinänen et al., 2018; Futia and Rinchard, 2019; Vuorinen et al., 2021). The egg THIAM concentration has therefore been used as the indicator in predicting and estimating the proportion of M74 females and female-specific M74 mortality percentage of yolk-sac fry (Keinänen et al., 2014; Vuorinen et al., 2021). The incipient M74 mortality of salmon yolk-sac fry is estimated to occur at an egg THIAM concentration of 0.71 nmol g^{-1}, whereas at a concentration of <0.22 nmol g^{-1}, 100% mortality is expected (Vuorinen et al., 2021). At these THIAM concentrations, the TotTh concentrations in the eggs of salmon were 1.35 nmol g^{-1} and 0.76 nmol g^{-1} respectively, which were considerably smaller, due to the lower TPP concentration in salmon, than the TotTh concentrations (1.958–9.649 nmol g^{-1}) in the lamprey eggs.

According to the THIAM vs. yolk-sac fry mortality model for salmon (Vuorinen et al., 2021), based on the THIAM concentration of lamprey eggs, all the offspring of 25% of lamprey females would have succumbed after hatching, and 98% of offspring would have at least displayed M74 symptoms, and a proportion of those would possibly have died. The respective TotTh model compiled for salmon (Vuorinen et al., 2021) is not relevant for lampreys because TPP consists of a considerably higher proportion of TotTh in lampreys than in salmon. Overall, the TPP of the egg thiamine components of salmonines has a weak relationship with the proportion of thiamine-deficient females and female-specific M74 and TDC mortality percentage of yolk-sac fry, and TotTh therefore also has a weaker relationship than THIAM (Czesny et al., 2012; Keinänen et al., 2018; Vuorinen et al., 2021).

The yolk reserves of the lamprey larvae are assimilated in a few days, while the yolk-sac phase of the salmon lasts 6–8 weeks at the same temperatures. The need for thiamine is therefore not as high for lampreys as for salmon during the endogenous feeding period. Mild thiamine deficiency may not cause M74 symptoms and mortality in salmon offspring until toward the end of the several-week yolk-sac phase (Lundström et al., 1999; Keinänen et al., 2014). For salmon, among the same offspring group, a proportion of yolk-sac fry may die, but the rest can survive and be lively (Keinänen et al., 2014). The eggs of the two groups of lamprey females were incubated together and not female-specifically, and a large proportion of the eggs was lost due to technical problems such as sudden changes in waterflow. The sparse hatched larvae were in poor condition and did not survive.

4.8. No diseases detected

No contagious bacterial or viral diseases were detected in the lampreys, but MORIB lampreys had chronic gill injuries and extensive bacteria cover on their gills. However, this was hardly the primary cause of the deaths, because it is quite common that bacteria rapidly increase in the gills of the weak and moribund fish on the bottom of a rearing tank. In the fall after heavy rains, the pH of the River Perhonjoki water can be low, and its metal concentrations may increase according to monitoring by the ELY Centre South Ostrobothnia (Sutela et al., 2012). However, if a low pH had injured the gills of the lampreys, they would already have died in the fall. It therefore seems the lampreys caught in the fall of 2014 had not been exposed to poor water quality during catching and keep netting. The gills of grayling (*Thymallus thymallus*) that were exposed to Al and Fe at pH 5.5 for a week and then allowed to recover in water with a neutral pH without metals recovered almost completely within a week (Peuranen et al., 2003). The observed gill injuries in the moribund lampreys at the hatchery were therefore not a result of a low pH during catching and holding in keepnets but was apparently related to secondary infections.

4.9. Preliminary trial on thiamine treatment of pre-spawning lampreys, and future work

In the following lamprey reproductive period, in 2015–2016, a preliminary thiamine treatment experiment was conducted to avoid thiamine deficiency-related losses of pre-spawning lampreys and to ensure that enough larvae were obtained for compensatory stockings. At the hatchery at the end of November 2015, thiamine (nominally 125 mg thiamine hydrochloride per kg body mass) was injected intraperitoneally into 600 specimens of the lampreys caught from the River Perhonjoki for compensatory production, and 600 specimens were untreated controls. The amount of injected thiamine corresponded at least to the largest amount with which salmonines have been treated (20–100 mg/kg body mass) when their thiamine status is assumed to be so poor that yolk-sac fry would die of thiamine deficiency (Koski et al., 1999, 2005; Futia et al., 2017). When stripping the eggs of the lamprey females in the following spring, the thiamine-injected ones had a wiry tonus, while the untreated fish were flabby. This may indicate that the thiamine status of the untreated females was poorer than that of the treated ones. However, there was no significant mortality in either group. In years past, the hatchery personnel at the Korpela hydropower plant (Risto Vikström and Eero Mäenpää, pers. comm.) observed that the largest lampreys were flabby when stripping the eggs in some years, but not every year. Large lampreys were often not stripped of the eggs at all because of their flabbiness and presumed low quality of eggs in order not to harm the incubation process.

Based on the preliminary trial, it was concluded that pre-spawning lampreys can be treated in the same way as salmonines to prevent deaths of females and offspring due to thiamine deficiency. This would need to be experimentally confirmed in the future. Safeguarding females is specifically important because there are usually fewer females than males in lamprey populations (Bartel et al., 2011). In the preliminary trial, the rearing tanks were also kept covered, which was thought to potentially save thiamine as a result of reduced swimming activity with less disturbances and thus less energy consumption and catabolism of fatty acids.

Recently, rearing methods and stockings of newly hatched larvae have been developed in the management of lamprey populations (Kujawa et al., 2018). Larval stocking is a more effective method than transferring adult lampreys over a dam in rivers that lack suitable spawning areas. Since thiamine deficiency can also weaken lamprey stocks, it must be taken into account in future research and development of farming and management methods of lampreys. What is worrying is that especially large females that would potentially produce the most offspring, as in other fish species (Merrett, 1994; Morita and Takashima, 1998; Rideout and Morgan, 2010), are most prone to dying of thiamine deficiency. The loss of the offspring of large females in the wild can cause a hereditary reduction in the size of the lampreys. There are indications of a decrease in the size of River Perhonjoki lampreys since the 1970s–1990s, which deserves to be investigated. Already in the early 1990s, the lampreys in various rivers of the Baltic Sea were the smallest in Finnish rivers (Bartel et al., 2011).

5. Conclusions

During the late winter—up to spawning in the late spring—lampreys, caught from a river flowing into the Gulf of Bothnia in the fall of 2014, suffered from lipid-related thiamine deficiency severe enough to cause the death of the largest and fattiest individuals. This is supported by the fact that it was mostly the largest and fattiest specimens that died, and the TotTh concentration in the liver was lower the fattier the lamprey, both in females and males. However, more of the females died of thiamine deficiency, because they were larger and fattier than the males, and their hepatic TotTh concentration was less than half that of the males. Of the females, the very smallest and leanest survived until spawning, because they had the best thiamine status, indicated by the highest hepatic TotTh concentration and highest THIAM concentration in the eggs. The concentrations of all other thiamine components in the eggs also correlated with the hepatic TotTh concentration, but THIAM had the strongest correlation. After the summers when the prey fish of lampreys are fatty and abundant, the reproduction of lampreys can be impaired due to thiamine deficiency. In nature, such poor recruitment is reflected in the number of ascending lampreys after several years at the earliest due to the long development time of the larvae in the river sediment. According to the preliminary experiment, thiamine deficiency caused by the abundant consumption of marine fatty prey fish and the resulting mortality of lamprey females during pre-spawning fasting, and their offspring, can possibly be prevented in hatcheries by injecting thiamine, especially into the largest lampreys, in the same way as with salmon. In the hatchery, the rearing tanks should be kept covered during the winter to help conserve the thiamine reserves of lampreys.

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Ethical approval

All procedures performed in the studies involving animals were in accordance with the ethical standards of the institution or practice in which the studies were conducted. The fish sampled for the study were taken from the commercial catches of professional fishermen and from routine aquaculture operations.

CRediT authorship contribution statement

Pekka J. Vuorinen: Conceptualization, Resources, Formal analysis, Data curation, Writing – original draft, Writing – review & editing, Visualization, Project administration. **Esa-Pekka Juntunen:** Conceptualization, Investigation, Resources, Writing – review & editing. **Juha Iivari:** Conceptualization, Investigation, Resources, Writing – review & editing. **Perttu Koski:** Conceptualization, Investigation, Resources, Validation, Data curation, Writing – review & editing, Project administration. **Soili Nikonen:** Investigation, Resources, Validation, Data curation, Writing – review & editing. **Mervi Rokka:** Resources, Validation, Data curation, Writing – review & editing. **Tiina Ritvanen:** Resources, Validation, Data curation, Writing – review & editing. **Jukka Pakkala:** Investigation, Resources, Data curation, Writing – review & editing. **Petri Heinimaa:** Resources, Writing – review & editing, Project administration. **Marja Keinänen:** Conceptualization, Resources, Formal analysis, Writing – original draft, Writing – review & editing, Visualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request

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Appendix A. Supplementary data

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.rsma.2023.102946.

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