Regional Studies in Marine Science 53 (2022) 102427

Contents lists available at ScienceDirect

Regional Studies in Marine Science

journal homepage: www.elsevier.com/locate/rsma

A screening study of relationships among concentrations of algal toxins, PFAS, thiamine deficiency and biomarkers in the European flounder from the southern Baltic Sea

Elin Dahlgren [a,*], Joacim Näslund [a], Lars Förlin [b], Lennart Balk [c], Lillemor Asplund [c], Dennis Lindqvist [c]

[a] Department of Aquatic Resources, Institute of Freshwater Research, Swedish University of Agricultural Sciences, Stångholmsvägen 2, 178 93 Drottningholm, Sweden
[b] Department of Biological and Environmental Sciences, University of Gothenburg, Box 463, 405 30, Gothenburg, Sweden
[c] Department of Environmental Science, Stockholm University, Svante Arrhenius väg 16, Stockholm, Sweden

ARTICLE INFO

Article history:
Received 18 May 2021
Received in revised form 3 January 2022
Accepted 2 May 2022
Available online 10 May 2022

Keywords:
Biomarkers
Hanöbukten
Fish health
Bromophenols
Hydroxylated polybrominated diphenyl ethers
Cyanotoxins
Perfluorinated alkyl substances
Vitamin B_1

ABSTRACT

In the disturbed Baltic Sea ecosystem, several animal species display failing health related to exposure to toxic compounds, reduced energy metabolism and immune system disorders. In order to investigate possible associations between fish health and exposure to chemicals affecting the energy metabolism and immune defence, the levels of algal toxins (bromophenols, hydroxylated polybrominated diphenyl ethers and nodularin), perfluorinated alkyl substances (PFAS) and thiamine (vitamin B_1) were determined in European flounder (*Platichthys flesus*). Several biomarkers indicating health status were examined in the fish, including ethoxyresorufin-O-deethylase (EROD), and activities of glutathione reductase, glutathione S-transferase and catalase, in addition to a large set of blood variables. The fish were collected from Hanöbukten in the south-western parts of the Baltic Sea in late August 2018. Regression analyses of algal toxins, PFAS and thiamine concentration displayed several significant associations with biomarkers associated with detoxification and liver function, immune system function and blood status of the fish.

1. Introduction

1.1. Fish health in the Baltic sea

Baltic Sea wildlife face a disturbed ecosystem with lingering effects of eutrophication (Murray et al., 2019), ongoing climate change (Reusch et al., 2018), intense fishing pressure (Ask and Svedäng, 2019), and emissions of hazardous substances (Lehtonen et al., 2017). Several fish species including Atlantic cod (*Gadus morhua*; 'cod'), Atlantic salmon (*Salmo salar*; 'salmon'), Eurasian perch (*Perca fluviatilis*; 'perch') and eelpout (*Zoarces viviparus*) display multifaceted health disorders affecting vital traits such as body condition (Casini et al., 2016; Engelhardt et al., 2020), immune defence (Förlin et al., 2019; Weichert et al., 2020) and reproduction (Åkerman and Balk, 1998; Förlin et al., 2019). The brackish waters of the southern Baltic Sea is particularly affected by multiple anthropogenic impacts (Korpinen et al., 2021). In the Hanöbukten (Hanö Bay) along the south east coast of Sweden (Fig. 1), European flounders (*Platichthys flesus;* 'flounder') seem to demonstrate episodic health disorders possibly indicating chemical exposure to toxic chemicals and disturbances related to energy metabolism and immune defence (Olsson et al., 2012). Studies of classic contaminants such as heavy metals, polychlorinated biphenyls (PCBs) and pesticides, brominated flame retardants (BFR), polyaromatic hydrocarbons (PAHs) and dioxins, furanes and dioxin-like PCBs in this region have not revealed any associations with the health disorders (Swedish Agency for Marine and Water Management, 2018). Instead, environmental stress seems to play an important role, as indicated in surveys of other coastal fish species (Hanson et al., 2020). In perch, recent findings reveal a possible association between elevated levels of naturally produced bioactive brominated compounds and disturbances of health biomarkers and detoxification (Förlin et al., 2019).

1.2. Algal toxins and thiamine

Environmental stress, caused by both abiotic and biotic factors, is known to induce production of secondary metabolites with toxic properties in primary producers, (Lehtimaki et al., 1997; Ross et al., 2006; Dahlgren et al., 2015) and affects the flow of

* Corresponding author.
E-mail address: elin.dahlgren@slu.se (E. Dahlgren).

https://doi.org/10.1016/j.rsma.2022.102427

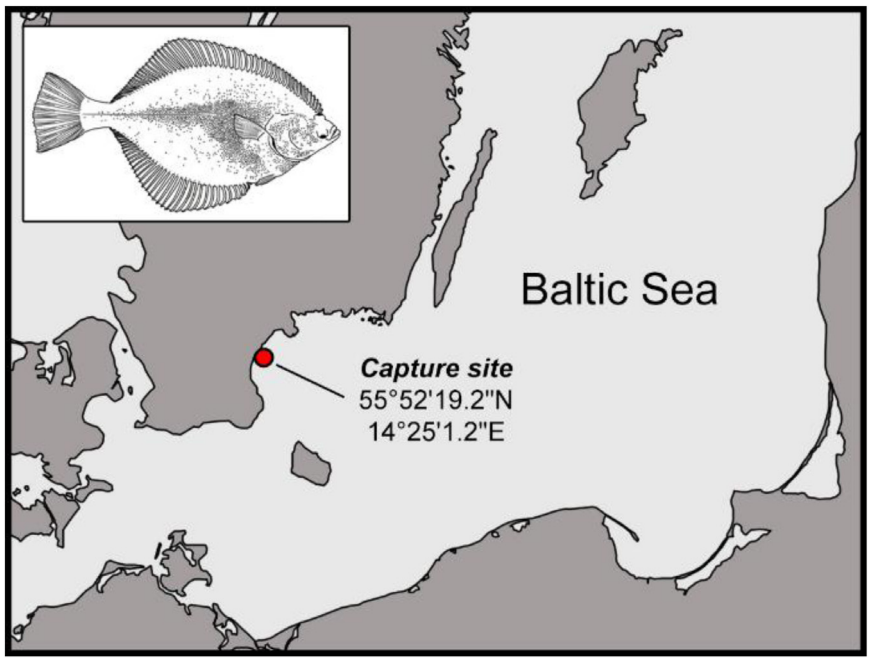

Fig. 1. Map of the Southern Baltic Sea showing the Hanö Bay capture site of European flounder.

nutrients produced at the base of the food web (Ejsmond et al., 2019; Majaneva et al., 2020). In the Baltic Sea, nodularin and toxic halogenated phenolic compounds, e.g. bromophenols (BPs) and hydroxylated polybrominated diphenyl ethers (OH-PBDEs), are produced by cyanobacteria and filamentous red- and brown algae respectively (Gribble, 2009; Malmvärn et al., 2008; Dahlgren et al., 2015). Nodularin, 2,4,6-tribromophenol (TBP) and 6-OH-BDE47 are detected throughout the Baltic food web (Sipiä et al., 2001, 2007, 2008; Lindqvist, 2016) and the concentrations of TBP and 6-OH-BDE47 are reportedly increasing (Faxneld et al., 2014). Several adverse effects have been associated with TBP and OH-PBDEs, in particular the congener 6-OH-BDE47, such as hormonal disturbances (Meerts et al., 2001), genotoxicity (Ji et al., 2011) and neurotoxicity (Dingemans et al., 2008). OH-PBDEs are also known to disrupt the oxidative phosphorylation (OXPHOS) and thereby affecting the energy metabolism (Legradi et al., 2014). Cyanotoxins, nodularin and microcystine are primarily hepatoxins (Honkanen et al., 1991; Ding et al., 2000). Nodularin is also known to cause neurotoxicity (Lehtonen et al., 2003), oxidative stress and developmental disorders (Chen et al., 2020).

Thiamine (vitamin B_1) is produced by primary producers and function as a micronutrient for the nervous system and as an essential cofactor for several enzymes involved in the metabolism of carbohydrates, fatty acids and amino acids (Whitfield et al., 2018). Lack of thiamine disrupt the energy metabolism via several modes of actions such as reduced ATP production, reduced oxidative phosphorylation (cellular NADH reduction), and reduced feeding (Balk et al., 2016; Harder et al., 2018). In the Baltic Sea, deficiency in thiamine has been detected in three animal classes: bivalves (Bivalvia), ray-finned fishes (Actinopterygii), and birds (Aves) (Balk et al., 2009, 2016; Mörner et al., 2017).

Even mild deficiency of thiamine, or low exposure to energy disrupting chemicals such as OH-PBDEs, may cause an equivalent effect to starvation in marine organisms and potentially also cause effects on the energy-demanding immune system and central nervous system. Emerging hazardous substances, like perfluorinated alkyl substances (PFASs), also reportedly cause effects on the immune system of marine organisms (Schultes et al., 2020; Liu and Gin, 2018). The present study screen for possible associations between biomarkers and concentrations of OXPHOS disruptors and immune defence suppressors in order to observe patterns on how these compounds may affect health biomarkers in flounder from Hanöbukten. The flounder is commonly used as a sentinel species in the Baltic Sea with numerous studies reporting biochemical and physiological responses (biomarkers) as means to measure effects, including combined effects of chemical pollutants (e.g. Dabrowska et al., 2014; Baršiene et al., 2014; Kopko and Dabrowska, 2018).

2. Materials and methods

2.1. Sampling

Flounders were caught with gill nets by local fishermen in Hanöbukten (Fig. 1) during the last week in August 2018. After being caught, fish were carefully disentangled from the net and kept for 2 to 3 days in corves situated at the sampling site, to allow the fish to recover from the fishing stress. For sampling of tissues, fish were killed by a sharp blow to the head, and blood was drawn by caudal venepuncture, using a heparin-prepared syringe. Fresh blood was used for measurement of the haematocrit, haemoglobin content, glucose levels and to produce blood smears for the blood cell count. Thereafter, blood was centrifuged for 90 s at 6000 G, and the plasma was separated and stored at −80 °C. After measuring the mass and total length, the fish was cut open and the bile collected from the gallbladder using a syringe. The liver was excised and weighed, and one piece was snap frozen in liquid nitrogen for preparation of measurement of enzyme activities. Fish were weighed after dissection for the somatic mass ('carcass mass'). The fish carcass was frozen at −20 °C and thereafter sent for storage to Swedish Environmental Specimen Bank at the Natural History Museum in Stockholm, Sweden. Ethical permission for the study was approved by the animal committee in Gothenburg, Sweden under the number 5.8. 18-02260/2018 and the identification number 001380.

2.2. Fish health

Liver samples were prepared as described in Förlin (1980). Biochemical analysis of biomarkers included; ethoxyresorufin-O-deethylase (EROD), activities of glutathione reductase (GR),

glutathione S-transferase (GST) and catalase (Cat). The numbers of immature red blood cells, thrombocytes, lymphocytes, and granulocytes and total amount of white blood cells were calculated from blood smears. The erythrocyte volume fractions (haematocrit) were estimated together with the haemoglobin and glucose concentrations in blood. Levels of the ions Na^+, K^+, Cl^- and Ca^{2+} in the blood plasma were determined. Further details on sample preparation and methodology of fish health biomarkers are provided in Electronic Supplementary Material (ESM).

2.3. Chemical analyses

Brominated phenolic compounds and thiamine were analysed at Stockholm University (Sweden), nodularin and microcystins at the University of Gdansk (Poland), and PFAS at the Örebro University. For details see ESM.

3. Data handling

Empirical parametrization of length–mass relationships of flounder often deviates from what is assumed in the commonly used 'Fulton' condition factor (K) formula (Froese, 2006; FishBase, 2019). Hence, residual body condition (RCF) was evaluated as the mass deviation (residual) from the empirical log–log relationship between total body mass (m_t) and total body length (L_t) in this study [model: $\log_e(m) \sim \log_e(L)$].

Gonad (ovary) mass was recalculated into a residual gonad index (RGI) by regressing a log–log relationship where gonad mass, m_g, is dependent on somatic mass, m_s [model: $\log_e(m_g) \sim \log_e(m_s)$]. Residuals for each individual, representing m_g deviance from expected values for a given m_s, were used in analyses. Liver mass, m_l, was recalculated into residual liver index (RLI), derived in the same way as RGI [model: $\log_e(m_l) \sim \log_e(m_s)$]. In contrast to the often used gonad- and liver somatic indices (GSI and LSI, respectively), these procedures avoid introducing bias caused by allometries in gonad- and liver mass; no size relationship remains in the residual data (Erickson et al., 1985).

All variables were graphically evaluated using histograms (Figures S1–S3) and data distributions with substantial positive skew (expected when data is strictly non-negative and have low mean value) were transformed using $\log_e$ (Glu) or $\log_e$ x + 1 (EROD, all PFAS variables, and all aromatic organohalogen compound (OHC) variables; the two latter groups of variables were transformed categorically as most of them showed skew). Non-phosphorylated thiamine and thiamine monophosphate in the liver (Thi_L, TMP_L) had positive skew (due to low, near-zero, levels of these compounds), but were retained on the original scale for consistency with other thiamine data; statistical analyses concerning relationships among thiamine and other variables are dependent on normality of residuals, not raw data. The reason for transforming variables was to reduce the statistical influence of the right distribution-tail in the correlation and regression analyses.

4. Statistical analyses

All biomarker and toxin variables were first screened for possible relationships with total body mass, using Pearson correlations. No significant relationships with body mass were indicated (α = 5%), see Supplement Table S1–3. Hence, body mass was not included as an independent variable in any subsequent statistical models.

Pearson correlation matrices, based on pairwise complete observations, were created separately for each functional group of biomarkers (for key to abbreviations, see Table 1): blood status and ion regulation (Hb, Ht, iRBC, $[K^+]$, $[Na^+]$, $[Ca^{2+}]$), immune defence (Lym, Gra, Thr, WBC), liver function (RLI, EROD, GR, GST, Cat), and growth and energy metabolism (RCF, RLI, RGI, Glu). The same procedure was applied to thiamine variables: non-phosphorylated thiamine (Thi), thiamine monophosphate (TMP), thiamine diphosphate (TDP), SumT (Thi + TMP + TDP), and toxicant groups: PFAS (PFOA, PFNA, PFOS, PFDA, PFUnDA), and organohalogen compounds [OHC (TBP, TBA, 6-OH-BDE49, 2′-OH-BDE68, 6-OH-BDE47, 6-OH-BDE90, 6-OH-BDE99, 2′-MeO-BDE68, 6-MeO-BDE47)]. These matrices provide details about covariation among compounds within each group of biomarkers and toxins.

Regression models were used to screen for effects of toxicants on biomarkers with biomarkers as dependent variables and toxicants as independent variables. Individual models for each combination of biomarker and toxicant were constructed. To screen for relationships between levels of toxicants and biomarker responses, standardized slope coefficients and their p-values (testing null-hypotheses of slope = 0) were calculated. Standardized slope estimates were obtained by mean-centring and scaling (1 standard deviation) the independent variable (*i.e.* the toxicant), using the jtools package for R (Long, 2020), and are used to make slope-estimates on different scales comparable.

Thiamine variables were tested as dependent variables in relation to both toxicants and biomarkers, using methods identical to that of the regressions for the biomarkers.

Since particular focus was directed to cyanobacterial toxins and thiamine variables, these are separately visualized and analysed in more detail. Nodularin from individual fish was compared with mean values from previous surveys in the Baltic Sea basins (Sipiä et al., 2001), to provide a view of the observed levels. Thiamine and its derivates were similarly visualized and also statistically analysed, comparing levels in the liver and brain of individual fish using paired t-tests (two-sided).

Since the aim of the analyses in general is screening, with the aim to indicate possible relationships, no p-value adjustments for multiple testing are applied. Such a procedure would defeat the purpose of screening for interesting correlational trends. However, due to the large number of tests, false positives (indication of effect when there is none in reality) are likely present in the results. Under the assumption of no false negatives and uniform distributions of p-values when there are no real effects, the expected number of false positives were calculated for suites of tests. All p-values between 0.1 and 1.0 were binned in 0.05 intervals, and the average of the number of cases within these 18 bins is considered as the expected number of false positives with $p < 0.05$. This procedure is similar to the underlying theory of false discovery rates (Benjamini and Hochberg, 1995), but focusing on estimating a theoretical number of false positives rather than correcting the p-values themselves. Interpretation of the results is focused on biological relevance of effect sizes and consistency in patterns of effects, rather than null-hypothesis significance itself.

Analyses and figures were made in R (R Core Team, 2020), using the packages stats (R Core Team, 2020), tidyverse (Wickham et al., 2019), and corrplot (Wei and Simko, 2017).

5. Result

5.1. Biological variables and indexes

Apart from the expected correlation between body length and mass, no correlations were found between variables indicating size and energy status (Fig. 2). Examination of the relationship between biological variables indicating blood status and immune function showed significant positive correlations among Hb, Ht, and Ca^{2+} (Fig. 2). All immune system variables were generally positively correlated (albeit non-significantly, $p < 0.1$, in a few cases) (Fig. 2). Out of the variables indicating liver function and detoxification only GR and GST demonstrated a significant correlation, with a negative relationship (Fig. 2).

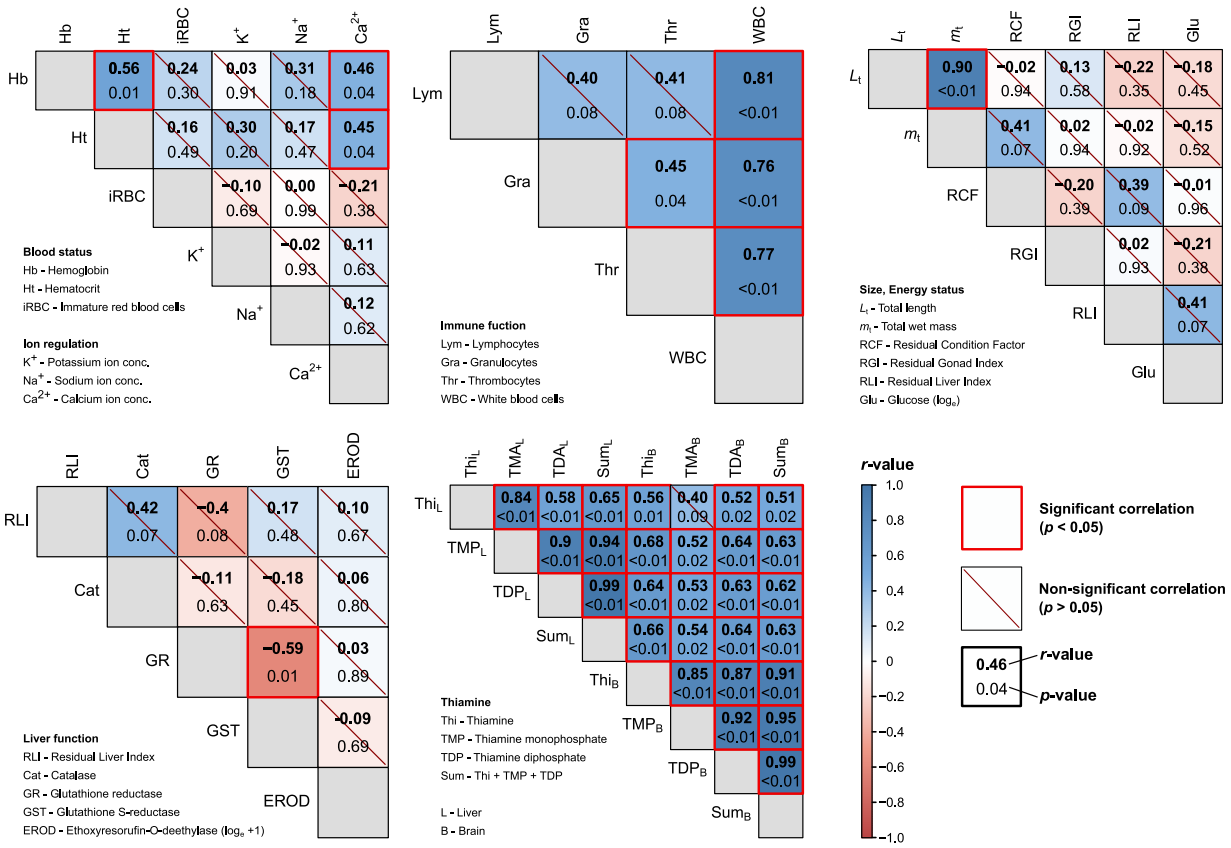

Fig. 2. Correlation matrices for biomarkers, grouped within their functional categories, and thiamine variables.

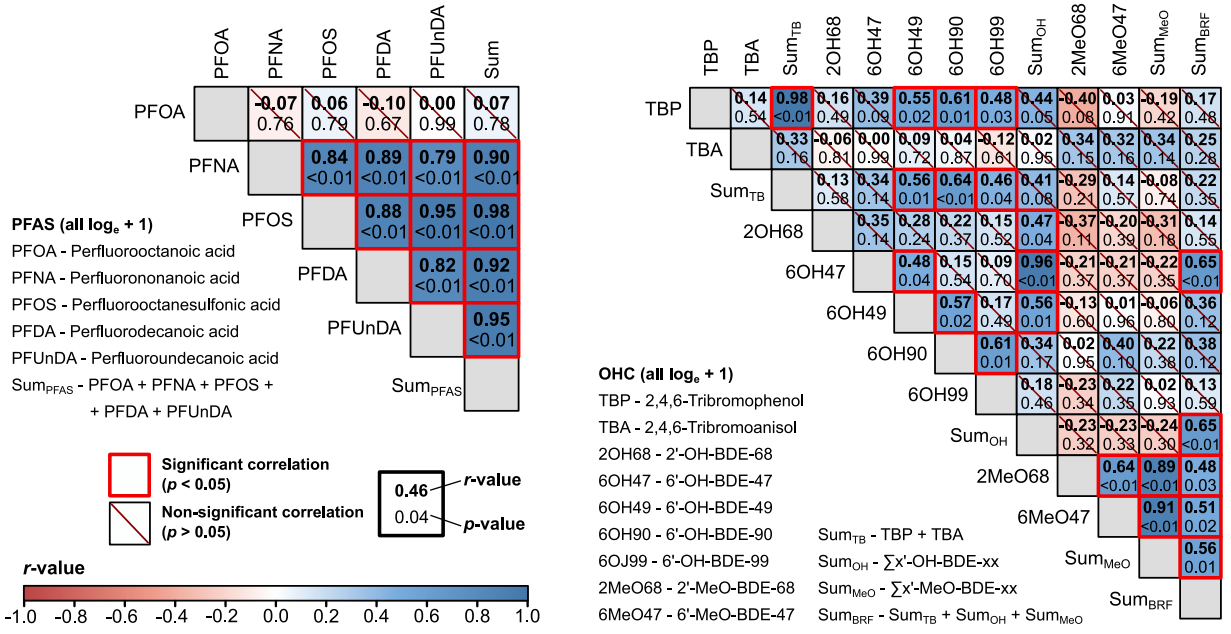

Fig. 3. Correlation matrices for toxicants; PFAS and OHC grouped separately.

5.2. Chemicals

The data of brominated phenolic compounds and MeO-PBDs, showed significant positive relationships between the congeners TBP vs. 6-OH-BDE49, TBP vs. 6-OH-BDE90, TBP vs. 6-OH-BDE99, 2-OH-BDE47 vs. 6-OH-BDE49, 2-OH-BDE49 vs. 6-OH-BDE90, and 2-OH-BDE90 vs. 6-OH-BDE99 (Fig. 3).

All measurements of thiamine, Thi, TMP and TDP in the liver and brain of the flounder demonstrated significant, positive correlations (Fig. 2). Sum of all thiamine variables were dominated by TDP values (see Fig. 6).

Except for PFOA (which was of lowest concentration of all quantified PFAS), all of the various PFAS demonstrated strong positive correlations within the fish (Fig. 3).

Table 1
Measured variables, with abbreviations, units, sample size (N), sample mean and standard mean deviation (SD), and functional category. PFAS = perfluorinated alkylated substances; OHC = organohalogen compounds; g_{dm} = gram dry mass; g_{wm} = gram wet mass.

Biomarker variables	Abbreviation	Unit	N	Mean ± SD	Functional category
Total body length	L_t	mm	20	318 ± 26	Size
Total wet mass	m_t	g	20	345 ± 63	Size
Soma wet mass	m_s	g	20	335 ± 61	Size
Gonad wet mass	m_g	g	20	10.6 ± 3.0	Reproduction, Energy status
Liver wet mass	m_l	g	20	10.7 ± 3.0	Liver function, Energy status
Glucose	Glu	mmol/l	20	4.11 ± 1.13	Energy status
Haemoglobin	Hb	g/l	20	64.3 ± 9.3	Blood status
Haematocrit	Ht	%	20	23.1 ± 3.0	Blood status
Immature red blood cells	iRBC	%	20	0.89 ± 0.19	Blood status
Potassium ion concentration	K^+	mmol/l	20	3.28 ± 0.41	Ion regulation
Sodium ion concentration	Na^+	mmol/l	20	147 ± 5	Ion regulation
Calcium ion concentration	Ca^{2+}	mmol/l	20	1.11 ± 0.09	Ion regulation
Lymphocytes	Lym	%	20	1.28 ± 0.42	Immune defence
Granulocytes	Gra	%	20	0.91 ± 0.32	Immune defence
Thrombocytes	Thr	%	20	1.11 ± 0.33	Immune defence
White blood cells	WBC	%	20	3.31 ± 0.84	Immune defence
Ethoxyresorufin-O-deethylase	EROD	pmol/mg protein x min	20	37.9 ± 29.7	Liver function, Detoxification
Glutathione reductase	GR	nmol/mg protein x min	20	9.69 ± 2.73	Liver function, Detoxification
Glutathione S-transferase	GST	µmol/mg protein x min	20	0.46 ± 0.08	Liver function, Detoxification
Catalase	Cat	µmol/mg protein x min	20	180 ± 52	Liver function, Detoxification
Thiamine variables					
Thiamine (liver)	Thi_L	nmol/g_{wm}	19	0.004 ± 0.011	Thiamine
Thiamine monophosphate (liver)	TMP_L	nmol/g_{wm}	19	0.90 ± 0.56	Thiamine
Thiamine diphosphate (liver)	TDP_L	nmol/g_{wm}	19	4.54 ± 1.93	Thiamine
Thiamine (brain)	Thi_B	nmol/g_{wm}	20	1.08 ± 0.53	Thiamine
Thiamine monophosphate (brain)	TMP_B	nmol/g_{wm}	20	2.65 ± 0.71	Thiamine
Thiamine diphosphate (brain)	TDP_B	nmol/g_{wm}	20	11.2 ± 2.5	Thiamine
Measured toxicant variables					
Nodularin	Nod	ng/g_{dm}	20	8.30 ± 12.0	Cyanobacterial toxin
Microcystin	Mcy	ng/g_{dm}	20	[a]	Cyanobacterial toxin
Perfluorooctanoic acid	PFOA	ng/g_{wm}	20	0.012 ± 0.013	PFAS
Perfluorononanoic acid	PFNA	ng/g_{wm}	20	0.050 ± 0.061	PFAS
Perfluorooctanesulfonic acid	PFOS	ng/g_{wm}	20	0.116 ± 0.091	PFAS
Perfluorodecanoic acid	PFDA	ng/g_{wm}	20	0.038 ± 0.044	PFAS
Perfluoroundecanoic acid	PFUnDA	ng/g_{wm}	20	0.041 ± 0.034	PFAS
Perfluorododecaoic acid	PFDoDA	ng/g_{wm}	20	[b]	PFAS
PFAS (other)[a]			20		
2,4,6-Tribromophenol	TBP	pg/g_{wm}	20	591 ± 432	OHC
2,4,6-Tribromoanisole	TBA	pg/g_{wm}	20	242 ± 80	OHC
2′-OH-BDE-68	2OH68	pg/g_{wm}	20	15.7 ± 3.6	OHC
6-OH-BDE-47	6OH47	pg/g_{wm}	20	43.2 ± 26.2	OHC
6′-OH-BDE-49	6OH49	pg/g_{wm}	19	7.41 ± 1.56	OHC
6-OH-BDE-90	6OH90	pg/g_{wm}	18	12.3 ± 3.0	OHC
6-OH-BDE-99	6OH99	pg/g_{wm}	20	12.9 ± 2.7	OHC
2′-MeO-BDE-68	2MeO68	pg/g_{wm}	20	31.7 ± 13.7	OHC
6-MeO-BDE-47	6MeO47	pg/g_{wm}	20	21.1 ± 15.7	OHC

[a]All samples below detection limit. PFAS substances below limits of detection were: PFBA, PFPeA, PFBS, PFBS, PFHxA, PFHpA, PFHpA, PFPeS, PFHxS, PFHpS, PFOA, PFNA, PFOS, PFDA, PFUnDA, PFNS, PFDS, PFDoDA, PFTrDA, PFDoDS, PFTDA, PFHxDA, PFOcDA, 4:2-FTSA, 6:2-FTSA, 8:2-FTSA, 5:3-FTCA, 6:2-FTUCA, 8:2-FTUCA, 10:2-FTUCA, 11ClPF3OUdS451, 9ClPF3ONS351.
[b]All but three samples below detection limit, not further analysed.

5.3. Relationships among biological indicators, biomarkers, and contaminants

In pairwise correlation analyses, several significant relationships were found between the various chemical toxins. In analyses of methoxylated and hydroxylated polybrominated diphenyl ethers, some compounds showed a significant relationship with biomarkers (Fig. 4, Fig S4). 2,4,6-tribromoanisole (TBA) was associated with biomarkers indicating liver function and detoxification (GST and EROD) and TBP with biomarkers indicating effects on the immune system (Lym and WBC). In addition, 6-OH-BDE47 demonstrated a negative association with residual gonad index.

Seven out of 30 analysed PFAS compounds were detected in the samples, dominated by perfluorononanoic acid (PFNA) which was present in all samples. *Perfluorooctane sulfonate* (PFOS) and perfluoroundecanoic acid (PFUnDA) were detected in 19 and 17 of the samples, respectively. Concentrations varied with an order of magnitude, with the highest levels detected for PFOS in muscle tissue at 0.36 ng g_{wm}^{-1}. The summarized values ranged between 0.02 and 0.95 ng g_{wm}^{-1} in muscle tissue. Regression analyses (Fig. 4, Fig. S4) demonstrated that several PFAS, including $\sum$ PFAS were negatively associated with granulocytes. PFOS and PFUnDA were significantly positively associated with immature red blood cells.

Nodularin was detected in 50% of the fish. Only two fish individuals had levels of nodularin ≥35 ng g_{dm}^{-1}, and none had levels over 50 ng g_{dm}^{-1} (Fig. 7). Nodularin was significantly positive associated to lymphocytes (Fig. 4, Fig. S4). Microcystins were not detected in any (0%) of the samples.

Concentrations of thiamine displayed significant associations with several biomarkers indicating fish health (summarized in Fig. 5; Fig S5). In regression analyses of thiamine and its derivates TMP and TDP, as dependent on body levels of toxicants, a few consistent patterns emerged. PFUnDA was positively associated with all thiamine variables, both in the liver and in the brain (all $p < 0.05$, except Thi_L with $p < 0.1$; Fig. 5A). 2′-OH-BDE68 was indicated to have a positive relationship with levels of Thi_L and

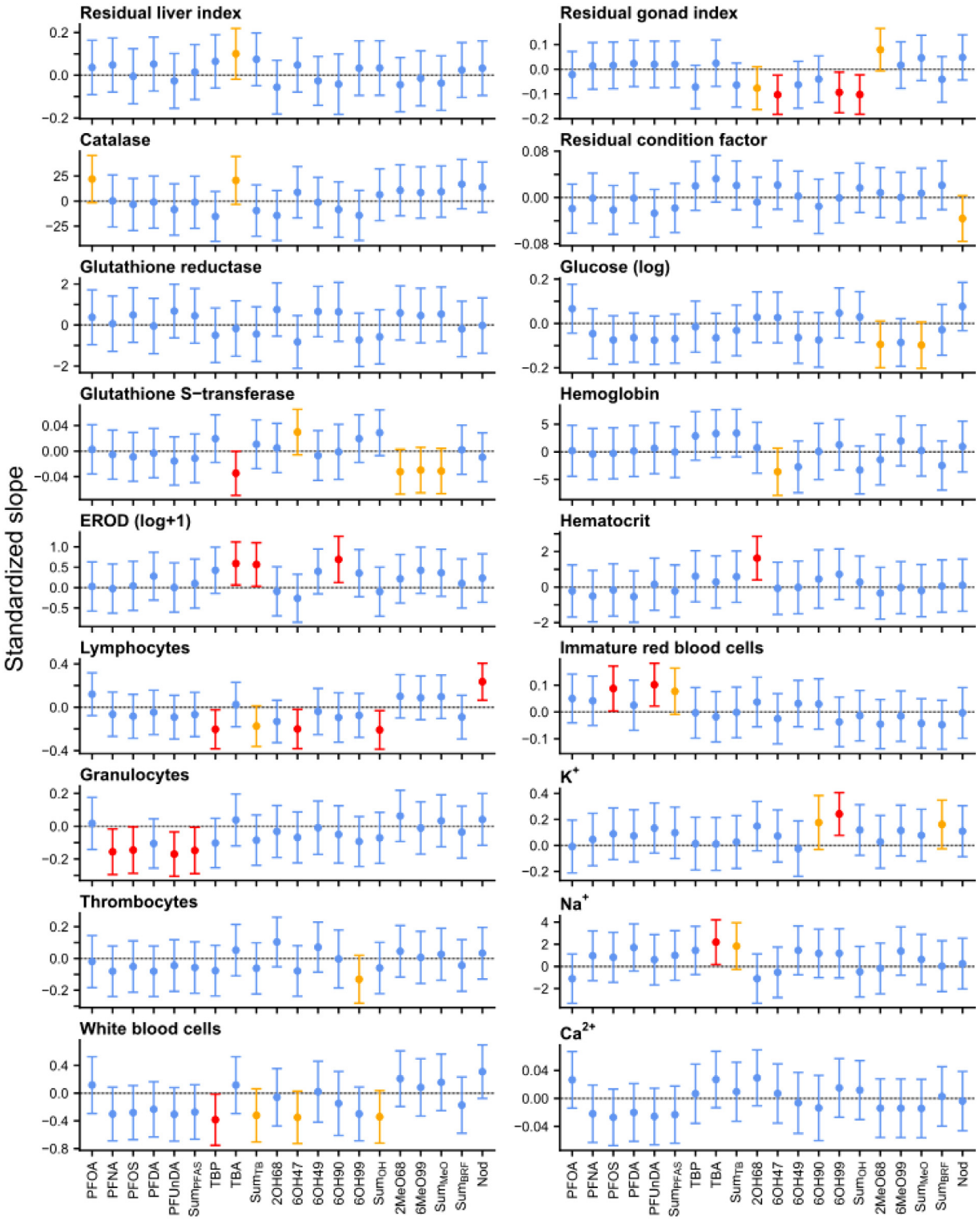

Fig. 4. Summary of regression analyses showing standardized slope estimates (with 95% confidence intervals) for regressions of biomarkers as dependent on levels of toxicants in the body of mature female European flounders, *Platichthys flesus*. Positive and negative values denote positive and negative relationships, respectively. Statistical significance is colour-coded as follows, red: $p < 0.05$; orange: $0.05 < p < 0.10$; blue: $p > 0.10$. Slopes are standardized by mean centring and scaling (1 SD) of the dependent variable in each analysis, to make the strength of the relationship visually comparable across analyses. Individual, non-standardized regressions for analyses with $p < 0.05$ are visualized in Fig. S4 (supplement). Key to abbreviations is found in Table 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

TMP_L ($p < 0.05$), and TDP_L appeared to be similarly influenced ($p < 0.1$) (Fig. 5A). However, this association was not detected in brain samples (Fig. 5A). 6-OH-BDE90 was indicated to have a positive relationship with TDP_L (also reflected in the sum of all liver thiamine variables; $p < 0.05$), but no other variables showed a similar relationship with this particular brominated phenol.

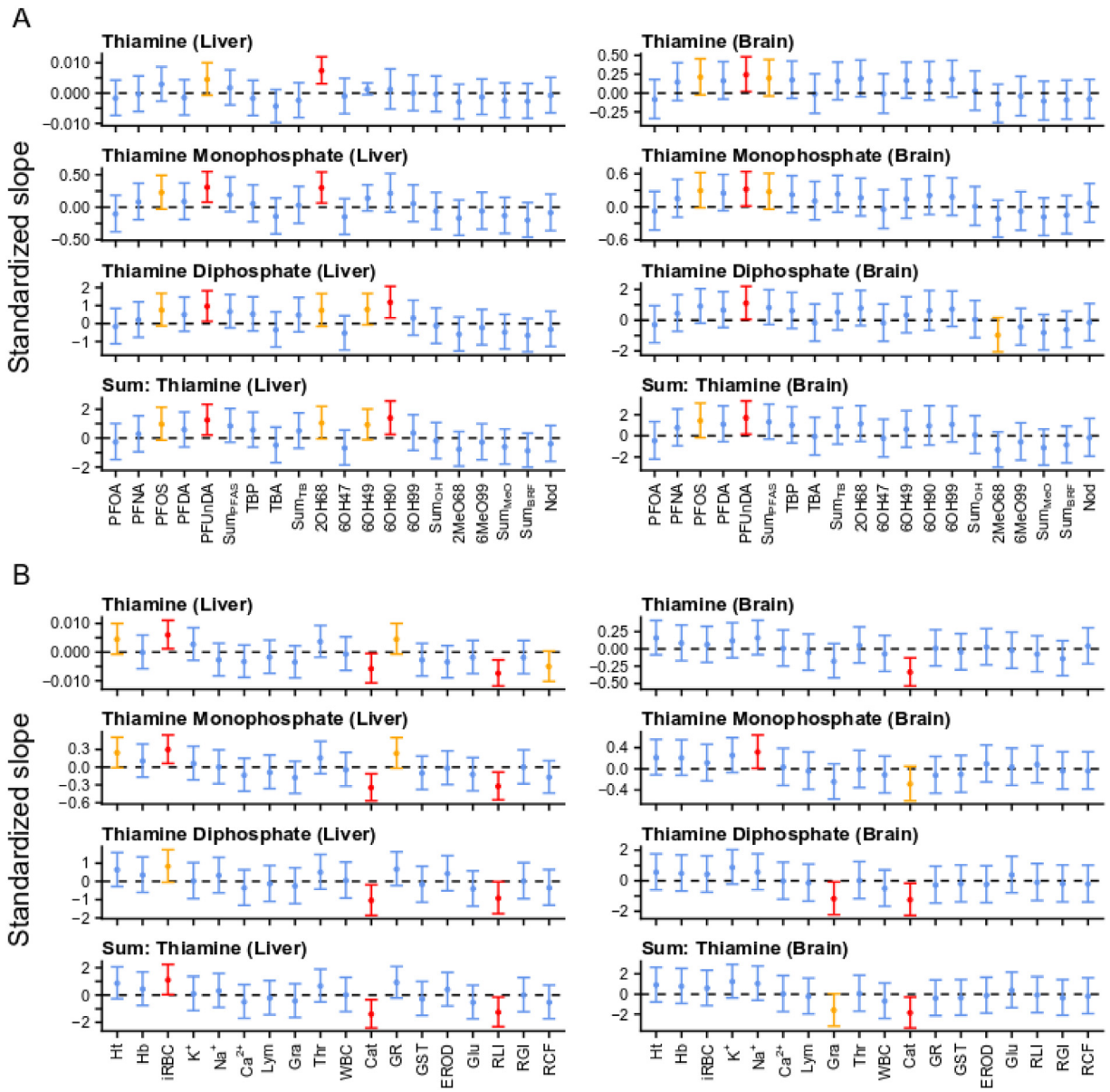

Fig. 5. Standardized slope estimates (with 95% confidence intervals) for regressions of thiamine variables as dependent on (A) toxicants, and (B) biomarkers in the body of mature female European flounders, *Platichthys flesus*. Positive and negative values denote positive and negative relationships, respectively. Statistical significance is colour coded as follows, red: $p < 0.05$; orange: $0.05 < p < 0.10$; blue: $p > 0.10$. Slopes are standardized by mean centring and scaling (1 SD) of the dependent variable in each analysis, to make the strength of the relationship visually comparable across analyses. Individual, non-standardized regressions for analyses with $p < 0.05$ are visualized in Fig. S5 (supplement). Key to abbreviations is found in Table 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Three biomarkers seemed to be associated to thiamine levels. Liver (but not brain) thiamine variables were positively associated to iRBC (all $p < 0.05$, except TDP_L with $p < 0.1$) and negatively associated to relative liver size (all $p < 0.05$) (Fig. 5B). All thiamine variables were negatively associated with catalase (all $p < 0.05$, except TMP_B with $p < 0.1$). In addition to general patterns, significant regressions indicated TMP_B as associated with Na^+ (positive effect) and TDP_B as associated with Gra (Fig. 5B).

Levels of thiamine in the liver were in all cases (all variables for all fish individuals) lower than in the brain, rendering all comparisons of differences (paired *t*-tests) statistically clear (all $p < 0.001$) (Fig. 6). Levels of thiamine in the liver (Thi_L) were very low (Fig. 6; Fig. S2; Table 1).

5.4. Expected number of false positives

The expected numbers of false positives ($p < 0.05$) were estimated to be 1.7 for biomarker correlations (Fig. 2), 3.3 for toxicant correlations (Fig. 3), 17.6 for biomarker regressions (Fig. 3), and 10.7 for thiamine regressions (Fig. 5; analyses on sums of thiamine variables not included in calculations, as they largely reflect TDP, see Fig. 6). The assumption of no false negatives with $p > 0.1$ likely makes these estimates conservative.

6. Discussion

In the present study, regression analyses of toxic chemicals and thiamine displayed significant associations with several

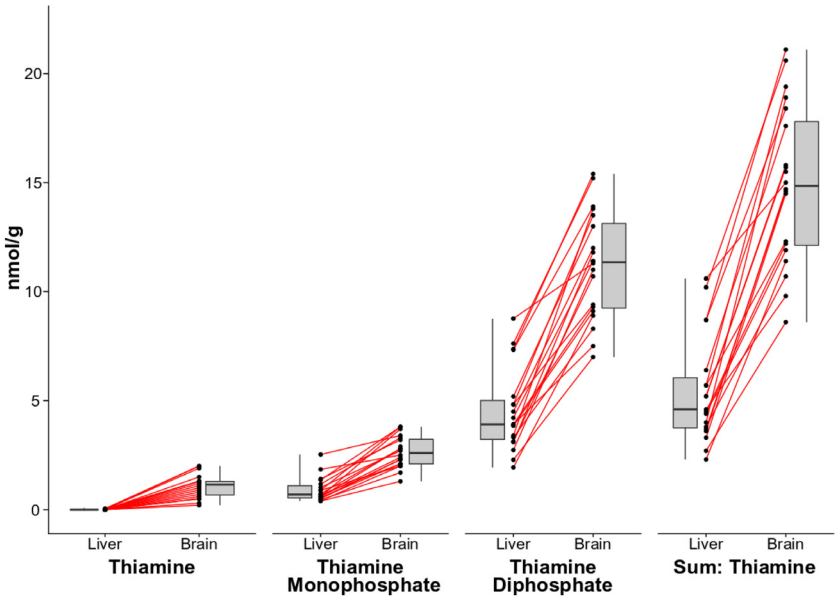

Fig. 6. Levels of thiamine, thiamine monophosphate, and thiamine diphosphate in liver and brain tissue of female mature European flounder *Platichthys flesus* from Hanöbukten (August 2018). Data is visualized as boxplots where the box delimits the second and third quartile, with median as a bold horizontal line; whiskers represent the first and fourth quartile (lower whisker not visible). Raw-data points originating from the same individual (liver vs. brain samples) are connected with a red line. The right-most plot show the sum of all three thiamine variables collated.

biomarkers associated with fish health. As such, the results provide a guide for future investigations into these relationships, which could help to explain ecotoxicological issues in the Baltic Sea ecosystem.

Health parameters

Within the Swedish National Monitoring Program (Sandström et al., 2005), an ongoing deterioration of fish health in response to exposure to chemical exposure has been suggested in coastal perch and eelpout collected from reference sites with no known sources of pollution (Förlin et al., 2019; Hanson et al., 2020). Disturbance of the immune system and blood status is indicated by increasing/deviating number of white blood cells (WBC) and immature red blood cells (iRBC). Health disorders cannot presently be explained by the presence of well-known chemical pollutants measured in relation to monitoring of biomarkers (Mustamäki et al., 2020). Recent findings in perch, collected at a site characterized by no or minor local anthropogenic influences, indicated impaired health based on gene-expressions related to immune and oxidative stress (Förlin et al., 2019). Higher concentrations of naturally produced bioactive brominated compounds, such as brominated indoles and carbazoles were related to higher expression of the membrane transporter (MATE) and a detoxification enzyme COMT (Förlin et al., 2019). Our study strengthens the notion that not only anthropogenic contaminants constitutes hazards to the marine environment; naturally produced compounds within the aquatic environment also need to be considered.

Concentrations of algal toxins, thiamine and PFAS in flounder

Concentrations of cyanotoxins detected in the muscle tissue of flounder were relatively low with microcystins being consistently below detection limits and nodularin ranging between 0–42.8 ng g_{dm}^{-1}. For nodularin, muscle concentrations up to 100 ng g_{dm}^{-1} have been detected in flounders caught from the western Gulf of Finland in September–October (Sipiä et al., 2000).

Out of the brominated compounds detected in flounder in this study, the plasma concentrations of 6-OH-BDE47 at 43.2 pg g_{wm}^{-1} is much lower than previously reported levels in perch, where whole fish concentrations of OH-BDE-47 were found up to 13 000 pg g_{wm}^{-1} during mid-August (Dahlgren et al., 2016). In blood plasma, concentrations of nearly 2 ng g_{wm}^{-1} are reported in flounders from Askö in the Baltic proper, which was one order of magnitude higher than other investigated species such as herring (*Clupea harengus*), long-tailed duck (*Clangula hyemalis*) and grey seals (*Halichoerus grypus*) (Lindqvist, 2020). The highest concentrations of OH-PBDEs in the Baltic Sea are reported in blue mussels (*Mytilus edulis*) Löfstrand et al., 2011. As blue mussels constitute a significant part of the diet for flounder in the Baltic Sea (Borg et al., 2014), they are likely to be exposed to high concentrations. In general, OH-PBDEs as well as nodularin display large seasonal variation, with peak concentrations in mussels during June to July (Mazur-Marzec et al., 2007; Löfstrand et al., 2011). The relatively low concentrations of algal toxins in flounder in the present study is likely a consequence of that the sampling was conducted during the latter part of the summer.

Concentrations of $\sum$ PFAS in this study at 0.3 ng g_{wm}^{-1} in muscle tissue is one order of magnitude lower than previously reported in flounder PFAS ($\sum$ PFCs, PFAAs, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFDoA, PFTrA, PFTeA, PFHxS, PFHpS, PFOS, and PFDS) from the Gulf of Finland at 5.3 ng g_{wm}^{-1} (Järv et al., 2017). In the same study, concentrations of $\sum$ PFAS in flounder reportedly vary between geographical sites.

All thiamine variables were positively correlated (Fig. 2), indicating consistent patterns among individuals, where low levels of one variable signal low levels of another variable. The concentrations of thiamine detected in the flounders were generally low. Several investigations (Rindi et al., 1963; Balaghi and Pearson, 1966; Batifoulier et al., 2005; Balk et al., 2016) have advocated that the ratio of the liver and brain $\sum$ Thiamine concentration could be used as a biomarker for thiamine status in vertebrates. The rationale behind this biomarker is that the brain has higher priority than the liver to not decrease, to maintain stability, in thiamine concentration. The ratio has been 2–3 in non-thiamine-deficient specimens, and as low as below 1 in thiamine-deficient specimens. The observed ratio for flounder in this study was below 0.5, suggesting a thiamine deficiency. Note that the sums of all thiamine variables are dominated by TDP values (see Fig. 7).

Some of the OH-PBDEs had a significant, positive, association with liver concentrations of thiamine, which is likely a result of

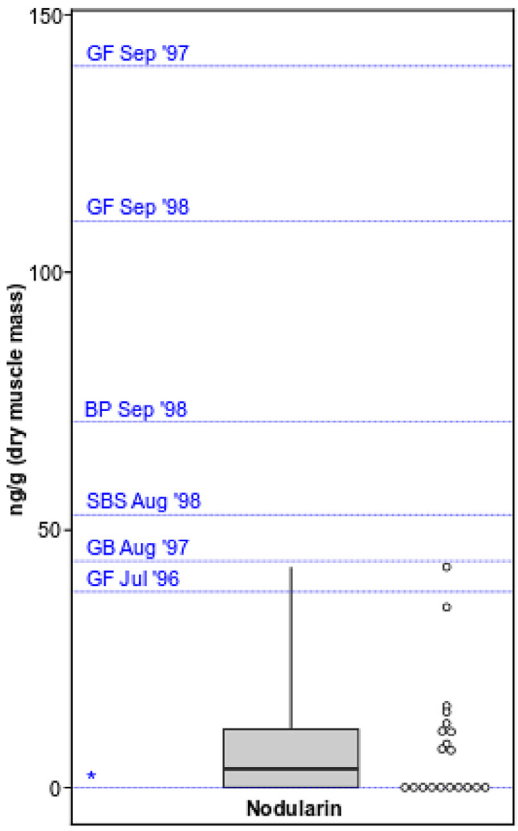

Fig. 7. Nodularin levels in muscle tissue of mature female European flounder *Platichthys flesus* in the Hanö bay area, southern Baltic Sea, collected August 2018. Data is visualized as a boxplot where the box delimits the second and third quartile, with median as a bold horizontal line; whiskers represent the first and fourth quartile (lower whisker not visible). Raw-data points (hollow circles) are presented to the right. Horizontal blue lines show, for reference, previously measured levels in liver tissue (dry mass; ELISA) from European flounder in the Baltic Sea, with month and year of the sampling (Sipiä et al., 2001): GF = Gulf of Finland; GB = Gulf of Bothnia; BP = Baltic Proper; SBS = southern Baltic Sea (SD 24). Asterisk (*) denote samplings with levels below the detection limit (Sipiä et al., 2001): GB Sep '98 (liver), GF Sep '97 (muscle), GB Aug '97 (muscle).

the shared origin (from primary producers) of these compounds. There was an overall strong, positive, association between the perflourinated compound PFUnDA and all measures of thiamine in the flounder, a relationship lacking explanation.

The observed associations between groups of biomarkers (detoxification and liver function, immune system and blood status) and chemical compounds demonstrate complex interactions including a mix of chemical pollutants with similar biological actions ('cocktail effects', Celander, 2011). The potential associations between different groups of biomarkers and compounds that are indicated in this screening require further investigations. Furthermore, additive and synergistic effects need to be evaluated. Possible links to oxidative stress is another area of future investigations.

6.1. False positives

The large number of tests applied in this study is likely to lead to Type I errors (false positives); *i.e.* erroneous rejection of the null-hypothesis of no effect. We argue that this is not a major problem in a screening study where all possible associations need to be indicated. Hence, we did not apply family-wise corrections for multiple testing. While possible causes for the associations are presented above, strict conclusions of effects relating to the patterns presented here should be avoided. Hence, our results should be viewed as indications of possible effects that should be followed up by confirmatory studies (unless the effect is already well established in previous studies).

6.2. Conclusions

In order to shed light on the cause of the deteriorating health of fish species in the Baltic Sea, it is important to include multiple chemical stressors, of natural as well as anthropogenic origin. Production and flow of compounds released by primary producers needs to be included when considering the potential cocktail of chemicals that affect the health of all aquatic organisms in the Baltic Sea.

CRediT authorship contribution statement

Elin Dahlgren, Dennis Lindqvist, Lars Förlin, Lennart Balk and Lillemor Asplund formulated and designed the study. Joacim Näslund developed the theory and performed the statistical computations. Elin Dahlgren, Dennis Lindqvist, Lars Förlin, Lennart Balk and Lillemor Asplund verified the analytical methods. All authors discussed the results and contributed to the final manuscript. Lars Förlin was in charge of fish collection and analyses of fish health biomarkers. Dennis Lindqvist was in charge of chemical analyses of brominated phenolic toxins and Lennart Balk for analyses of thiamine levels. Project administrations was coordinated by Elin Dahlgren who also wrote the manuscript with support from Dennis Lindqvist, Joacim Näslund, Lars Förlin, Lennart Balk and Lillemor Asplund.

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.

Acknowledgements

We would like to thank Henrik Dahlgren, Hanna Gustavsson and Jari Parkkonen for sample preparation and biomarkers analysis.

Funding sources

This work was supported by the Swedish Environmental Protection Agency, Vattenvårdsförbundet i västra Hanöbukten, Göte Borgströms foundation (to LB), the Department of Aquatic Resources, Swedish University of Agricultural Sciences (to ED and JN), and The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning, FORMAS (Dnr 2018-01571 to DL).

Appendix A. Supplementary data

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.rsma.2022.102427.

References

Åkerman, G., Balk, L., 1998. Descriptive studies of mortality and morphological disorders in early life stages of cod and salmon originating from the Baltic sea. Am. Fish. Soc. Symp. 21, 41–61.

Ask, L., Svedäng, H., 2019. En Näring i Nationens Tjänst – Utveckling av Fisket Och Fiskeriförvaltningen i Sverige (an Industry at the Nation's Service - Development of Fishing and Fisheries Management in Sweden). Havsmiljöinstitutets rapport 2019:7, Havsmiljöinstitutet, Gothenburg, p. 60, (in Swedish).

Balaghi, M., Pearson, W.N., 1966. Tissue and intracellular distribution of radioactive thiamine in normal and thiamine-deficient rats. J. Nutr. 89, 127–132.

Balk, L., Hägerroth, P.-Å., Åkerman, G., Hanson, M., Tjärnlund, U., Hansson, T., Hallgrimsson, G.T., Zebühr, Y., Broman, D., Mörner, H., 2009. Wild birds of declining European species are dying from a thiamine deficiency syndrome. Proc. Natl. Acad. Sci. USA 106, 12001–12006.

Balk, L., Hägerroth, P.-Å., Gustavsson, H., Sigg, L., Åkerman, G., Muñoz, Y.R., Honeyfield, D.C., Tjärnlund, U., Oliveira, K., Ström, K., McCormick, S.D., Karlsson, S., Ström, M., van Manen, M., Berg, A.-L., Halldórson, H.P., Strömquist, J., Collier, T.K., Börjeson, H., Mörner, T., 2016. Widespread episodic thiamine deficiency in northern hemisphere wildlife. Sci. Rep. 6, 38821.

Baršiene, J., Butrimavičiene, L., Grygiel, W., Lang, T., Michailovas, A., Jackūnas, T., 2014. Environmental genotoxicity and cytotoxicity in flounder (*Platichthys flesus*), herring (*Clupea harengus*) and Atlantic cod (*Gadus morhua*) from chemical munitions dumping zones in the southern Baltic sea. Mar. Environ. Res. 96, 56–67.

Batifoulier, F., Verny, M.-A., Besson, C., Demigne, C., Remesy, C., 2005. Determination of thiamine and its phosphate esters in rat tissues analyzed as thiochromes on a RP-amide C16 column. J. Chromatogr. B 816, 67–72.

Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc. Ser. B Stat. Methodol. 57, 289–300.

Borg, J.P.G., Westerbom, M., Lehtonen, H., 2014. Sex-specific distribution and diet of *Platichthys flesus* at the end of spawning in the northern Baltic sea. J. Fish Biol. 84, 937–951.

Casini, M., Käll, F., Hansson, M., Plikshs, M., Baranova, T., Karlsson, O., Lundström, K., Neuenfeldt, S., Gårdmark, A., Hjelm, J., 2016. Data from: Hypoxic areas, density-dependence and food limitation drive the body condition of a heavily exploited marine fish predator. Dryad Digit. Repos. http://dx.doi.org/10.5061/dryad.875fb.

Celander, M.C., 2011. Cocktail effects on biomarker responses in fish. Aquat. Toxicol. 105, 72–77.

Chen, X.-D., Liu, Y., Yang, L.-M., Hu, X.-Y., Jia, A.-Q., 2020. Hydrogen sulfide signaling protects chlamydomonas reinhardtii against allelopathic damage from cyanobacterial toxin microcystin-LR. Front. Plant Sci. 11, 1105.

Dabrowska, H., Kopko, O., Góra, A., Waszak, I., Walkusz-Miotk, J., 2014. DNA damage, EROD activity, condition indices, and their linkages with contaminants in female flounder (*Platichthys flesus*) from the southern Baltic sea. Sci. Tot. Environ. 496, 488–498.

Dahlgren, E., Enhus, C., Lindqvist, D., Eklund, B., Asplund, L., 2015. Induced production of brominated aromatic compounds in the alga *Ceramium tenuicorne*. Environ. Sci. Pollut. Res. 22, 18107–18114.

Dahlgren, E., Lindqvist, D., Dahlgren, H., Asplund, L., Lehtila, K., 2016. Trophic transfer of naturally produced brominated aromatic compounds in a Baltic sea food chain. Chemosphere 144, 1597–1604.

Ding, W.-X., Shen, H.-M., Ong, C.-N., 2000. Microcystic cyanobacteria extract induces cytoskeletal disruption and intracellular glutathione alteration in hepatocytes. Environ. Health Perspect. 108, 605–609.

Dingemans, M.M., de Groot, A., van Kleef, R.G., Bergman, Å., van den Berg, M., Vijverberg, H.P., Westerink, R.H., 2008. Hydroxylation increases the neurotoxic potential of BDE-47 to affect exocytosis and calcium homeostasis in PC12 cells. Environ. Health Perspect. 116, 637–643.

Ejsmond, M.J., Blackburn, N., Fridolfsson, E., Haecky, P., Andersson, A., Casini, M., Belgrano, A., Hylander, S., 2019. Modeling vitamin B_1 transfer to consumers in the aquatic food web. Sci. Rep. 9, 10045.

Engelhardt, J., Frisell, O., Gustavsson, H., Hansson, T., Sjöberg, R., Collier, T.K., Balk, L., 2020. Severe thiamine deficiency in eastern Baltic cod (*Gadus morhua*). PLoS One 15, e0227201.

Erickson, D.L., Hightower, J.E., Grossman, G.D., 1985. The relative gonadal index: an alternative for quantification of reproductive condition. Comput. Biochem. Phys. A 81, 117–120.

Faxneld, S., Helander, B., Bäcklin, B.-M., Moraeus, C., Roos, A., Berger, U., Egebäck, A.-L., Strid, A., Kierkegaard, A., Bignert, A., 2014. Biological Effects and Environmental Contaminants in Herring and Baltic Sea Top Predators. Rapport 6:2014, Swedish Museum of Natural History, Stockholm, p. 159.

FishBase, 2019. Length-weight parameters for *Platichthys flesus*. In: Froese, R., Pauly, D. (Eds.), FishBase V.12/2019. https://www.fishbase.org/.

Förlin, L., 1980. Effects of clophen A50, 3-methylcholanthrene, pregnenolone-16α-carbonitril and phenobarbital on the hepatic microsomal cytochrome P-450-dependen monooxygenase system in rainbow trout, salmo gairdneri, of different age and sex. Toxicol. Appl. Pharmacol. 54, 420–430.

Förlin, L., Asker, N., Töpel, M., Österlund, T., Kristiansson, E., Parkkonen, J., Haglund, P., Faxneld, S., Sturve, J., 2019. mRNA expression and biomarker response in perch at a biomonitoring site in the Baltic sea – possible influence of natural brominated chemicals. Front. Mar. Sci. 6, 316.

Froese, R., 2006. Cube law, condition factor and weight-length relationships: history, meta-analysis and recommendations. J. Appl. Ichthyol. 22, 241–253.

Gribble, G.W., 2009. Naturally Occurring Organohalogen Compounds-a Comprehensive Update. Springer-Verlag/Wien, Vienna.

Hanson, N., Larsson, Å., Parkkonen, J., Faxneld, S., Nyberg, E., Bignert, A., Ek Henning, H., Bryhn, A., Olsson, J., Karlson, A.M.L., Förlin, L., 2020. Ecological changes as a plausible explanation for differences in uptake of contaminants between European perch and eelpout in a coastal area of the Baltic sea. Environ. Toxicol. Pharmacol. 80, 103455. http://dx.doi.org/10.1016/j.etap.2020.103455.

Harder, A.M., Ardren, W.R., Evans, A.N., Futia, M.H., Kraft, C.E., Marsden, J.E., Richter, C.A., Rinchard, J., Tillitt, D.E., Christie, M.R., 2018. Thiamine deficiency in fishes: Causes, consequences, and potential solutions. Rev. Fish Biol. Fish. 28, 865–886.

Honkanen, R., Dukelow, M., Zwiller, J., Moore, R., Khatra, B., Boynton, A., 1991. Cyanobacterial nodularin is a potent inhibitor of type 1 and type 2A protein phosphatases. Mol. Pharmacol. 40, 577–583.

Järv, L., Kiviranta, H., Koponen, L., Rantakokko, P., Ruokojarvi, P., Radin, M., Roots, O., Simm, M., 2017. Persistent organic pollutants in selected fishes of the gulf of finland. J. Mar. Syst. 171, 129–133.

Ji, K., Choi, K., Giesy, J.P., Musarrat, J., Takeda, S., 2011. Genotoxicity of several polybrominated diphenyl ethers (PBDEs) and hydroxylated PBDEs, and their mechanisms of toxicity. Environ. Sci. Technol. 45, 5003–5008.

Kopko, O., Dabrowska, H., 2018. Variability of biological indices, biomarkers, and organochlorine contaminants in flounder (*Platichthys flesus*) in the gulf of gdańsk, southern Baltic sea. Chemosphere 194, 701–713.

Korpinen, S., Laamanen, L., Berström, L., Nurmi, M., Andersen, J.H., Haapaniemi, J., Harvey, E.T., Murray, C.J., Peterlin, M., Kallenbach, E., Klančnik, K., Stein, U., Tunesi, L., Vaughan, D., Reker, J., 2021. Combined effects of human pressures on Europe's marine ecosystems. Ambio http://dx.doi.org/10.1007/s13280-020-01482-x.

Legradi, J., Dahlberg, A.-K., Cenijn, P., Marsh, G.R., Asplund, L., Bergman, Å., Legler, J., 2014. Disruption of oxidative phosphorylation (OXPHOS) by hydroxylated polybrominated diphenyl ethers (OH-PBDEs) present in the marine environment. Environ. Sci. Technol. 48, 14703–14711.

Lehtimaki, J., Moisander, P., Sivonen, K., Kononen, K., 1997. Growth, nitrogen fixation, and nodularin production by two Baltic sea cyanobacteria. Appl. Environ. Microbiol. 63, 1647–1656.

Lehtonen, K.K., Bignert, A., Bradshaw, C., Broeg, K., Schiedek, D., 2017. Chemical pollution and ecotoxicology. In: Snoeijs-Leijonmalm, P., Schubert, H., Radziejewska, T. (Eds.), Biological Oceanography of the Baltic Sea. Springer, Dordrecht, pp. 547–587.

Lehtonen, K., Kankaanpää, H., Leiniö, S., Sipiä, V.O., Pflugmacher, S., Sandberg-Kilpi, E., 2003. Accumulation of nodularin-like compounds from the cyanobacterium *Nodularia spumigena* and changes in acetylcholinesterase activity in the clam *Macoma balthica* during short-term laboratory exposure. Aquat. Toxicol. 64, 461–476.

Lindqvist, D., 2016. Hydroxylated Polybrominated Diphenyl Ethers in the Baltiv Sea Biota (Doctoral thesis). Stockholm University, Stockholm.

Lindqvist, D., 2020. Screening of halogenated phenolic compounds in plasma and serum from marine wildlife. Int. J. Environ. Sci. Technol. 17, 2177–2184.

Liu, C., Gin, K.Y.H., 2018. Immunotoxicity in green mussels under perfluoroalkyl substance (PFAS) exposure: Reversible response and response model development. Environ. Toxicol. Chem. 27, 1138–1145.

Löfstrand, K., Liu, X., Lindqvist, D., Jensen, S., Asplund, L., 2011. Seasonal variations of hydroxylated and methoxylated brominated diphenyl ethers in blue mussels from the Baltic sea. Chemosphere 84, 527–532.

Long, J., 2020. jtools: Analysis and presentation of social scientific data. CRAN: https://cran.r-project.org/web/packages/jtools/.

Majaneva, S., Fridolfsson, E., Casini, M., Legrand, C., Lindehoff, E., Margonski, P., Majaneva, M., Nilsson, J., Rubene, G., Wasmund, N., Hylander, S., 2020. Deficiency syndromes in top predators associated with large-scale changes in the Baltic sea ecosystem. PLoS One 15, e0227714.

Malmvärn, A., Zebühr, Y., Kautsky, L., Bergman, Å., Asplund, L., 2008. Hydroxylated and methoxylated polybrominated diphenyl ethers and polybrominated dibenzo-p-dioxins in red alga and cyanobacteria living in the Baltic sea. Chemosphere 72, 910–916.

Mazur-Marzec, H., Tyminska, A., Szafranek, J., Plinski, M., 2007. Accumulation of nodularin in sediments, mussels, and fish from the gulf of gdansk, southern Baltic sea. Environ. Toxicol. 22, 101–111.

Meerts, I., Letcher, R.J., Hoving, S., Marsh, G., Bergman, A., Lemmen, J.G., van der Burg, B., Brouwer, A., 2001. In vitro estrogenicity of polybrominated diphenyl ethers, hydroxylated PDBEs, and polybrominated bisphenol A compounds. Environ. Health Perspect. 109, 399–407.

Mörner, T., Hansson, L., Berg, A.L., Muñoz, Y.R., Gustavsson, H., Mattson, R., Balk, L., 2017. Thiamine deficiency impairs common eider (*Somateria mollissima*) reproduction in the field. Sci. Rep. 7, 14451.

Murray, C.J., Müller-Karulis, B., Carstensen, J., Conley, D.J., Gustafsson, B.G., Andersen, J.H., 2019. Past, present and future eutrophication status of the Baltic sea. Front. Mar. Sci. 6, 2.

Mustamäki, N., Franzén, F., Persson, S., Tollerz Bratteby, U., Tärnlund, S., Pettersson, M., Olsson, J., Förlin, L., Larsson, Å., Parkkonen, J., Faxneld, S., Sköld, M., 2020. Faktablad från integrerad kustfiskövervakning 2020:1. (in Swedish) Availabe at: https://www.slu.se/faktablad-kustfisk.

Olsson, J., Bergström, L., Gårdmark, A., 2012. Abiotic drivers of coastal fish community change during four decades in the Baltic sea. ICES J. Mar. Sci. 69, 961–970.

R Core Team, 2020. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna.

Reusch, T.B.H., Dierking, J., Andersson, H.C., Bonsdorff, E., Carstensen, J., Casini, M., Czajkowski, M., Hasler, B., Hinsby, K., Hyytiäinen, K., Johannesson, K., Jomaa, S., Jormalainen, V., Kuosa, H., Kurland, S., Laikre, L., MacKenzie, B.R., Margonski, P., Melzner, F., Oesterwind, D., Ojaveer, H., Refsgaard, J.C., Sandström, A., Schwarz, G., Tonderski, K., Winder, M., Zandersen, M., 2018. The Baltic sea as a time machine for the future coastal ocean. Sci. Adv. 4, eaar8195.

Rindi, G., de Giuseppe, L., Ventura, U., 1963. Distribution and phosphorylation of oxythiamine in rat tissues. J. Nutr. 81, 147–154.

Ross, C., Santiago-Vázquez, L., Paul, V., 2006. Toxin release in response to oxidative stress and programmed cell death in the cyanobacterium *Microcystis aeruginosa*. Aquat. Toxicol. 78, 66–73.

Sandström, O., Larsson, Å., Andersson, J., Appelberg, M., Bignert, A., Ek, H., Förlin, L., Olsson, M., 2005. Three decades of Swedish experience demonstrates the need for integrated long-term monitoring of fish in marine coastal areas. Water Qual. Res. J. 40, 233–250.

Schultes, L., Sandblom, O., Broeg, K., Bignert, A., Benskin, J.P., 2020. Temporal trends (1981–2013) of per- and polyfluoroalkyl substances and total fluorine in Baltic cod (*Gadus morhua*). Environ. Toxicol. Chem. 39, 300–309.

Sipiä, V., Kankaanpää, H., Lahti, K., Carmichael, W.W., Meriluoto, J., 2001. Detection of nodularin in flounders and cod from the Baltic sea. Environ. Toxicol. 16, 121–126.

Sipiä, V., Kankaanpää, H., Meriluoto, J., Høisæter, T., 2000. The first observation of okadaic acid in flounder in the Baltic sea. Sarsia 85, 471–475.

Sipiä, V., Kankaanpää, H., Peltonen, H., Vinni, M., Meriluoto, J., 2007. Transfer of nodularin to three-spined stickleback (*Gasterosteus aculeatus* L.), herring (*Clupea harengus* L.), and salmon (*Salmo salar* L.) in the northern Baltic sea. Ecotoxicol. Environ. Safety 66, 421–425.

Sipiä, V.O., Neffling, M.-R., Metcalf, J.S., Nybom, S.M., Meriluoto, J.A., Codd, G.A., 2008. Nodularin in feathers and liver of eiders (*Somateria mollissima*) caught from the western gulf of finland in june–2005. Harmful Algae 7, 99–105.

Swedish Agency for Marine and Water Management, 2018. Miljön i Hanöbukten 2015-2017. Havs- och vattenmyndighetens rapport 2018:10, Swedish Agency for Marine and Water Management, Gothenburg, p. 81.

Wei, T., Simko, V., 2017. Corrplot: visualization of a correlation matrix. CRAN, https://cran.r-project.org/web/packages/corrplot/index.html.

Weichert, F.G., Axén, C., Förlin, L., Inostroza, P.A., Kamman, U., Welling, A., Sturve, J., Asker, N., 2020. A multi-biomarker study on Atlantic salmon (*Salmo salar* L.) affected by the emerging red skin disease in the Baltic sea. J. Fish Dis. http://dx.doi.org/10.1111/jfd.13288.

Whitfield, K.C., Bourassa, M.W., Adamolekun, B., Bergeron, G., Bettendorff, L., Brown, K.H., Cox, L., Fattal-Valevski, A., Fischer, P.R., Frank, E.L., Hiffler, L., Hlaing, L., M, Jefferds, M.E., Kapner, H., Kounnavong, S., Mousavi, M.P.S., Roth, D.E., Tsaloglou, M.-N., Wieringa, F., Combs, G.F., 2018. Thiamine deficiency disorders: diagnosis, prevalence, and a roadmap for global control programs. Ann. New York Acad. Sci. 1430, 3–43.

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L.D.A., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., 2019. Welcome to the Tidyverse. J. Open Source Softw. 4, 1686.