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Seasonal variation and species-specific concentrations of the essential vitamin B₁ (thiamin) in zooplankton and seston

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Abstract

Thiamin (vitamin B₁) is mainly produced by bacteria and phytoplankton and then transferred to zooplankton and higher trophic levels but knowledge on the dynamics of these processes in aquatic ecosystems is lacking. Hence, the seasonal variation in thiamin content was assessed in field samples of copepods and in pico-, nano- and micro-plankton of two size classes (0.7–3 µm and > 3 µm) collected monthly in the Baltic Sea during 3 years and in the Skagerrak during 1 year. Copepods exhibited species-specific concentrations of thiamin and *Acartia* sp. had the highest carbon-specific thiamin content, at both locations. Even members of the same genus, but from different systems contained different levels of thiamin, with higher thiamin content per specimen in copepods from the Skagerrak compared to congeners from the Baltic Sea. Furthermore, our results show that the small plankton (0.7–3 µm) had a higher carbon-specific thiamin content compared to the large (> 3 µm). Additionally, there was a large seasonal variation and thiamin content was highly correlated comparing the two size fractions. Finally, there was an overall positive correlation between thiamin content in copepods and plankton. However, for periods of high thiamin content in the two size fractions, this correlation was negative. This suggests a decoupling between thiamin availability in pico-, nano- and micro-plankton and zooplankton in the Baltic Sea. Knowledge about concentrations of this essential micronutrient in the aquatic food web is limited and this study constitutes a foundation for further understanding the dynamics of thiamin in aquatic environments.

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Introduction

Thiamin, formerly known as thiamine or aneurine (Combs 2012), is an essential vitamin that has various cellular functions, for instance as a co-enzyme involved in the cellular metabolism in the Krebs cycle (Manzetti et al. 2014; Sañudo-Wilhelmy et al. 2014). It is mainly produced by bacteria, phytoplankton and to some extent fungi in aquatic environments and is transferred to higher trophic levels, such as zooplankton, fish and birds. Similar to other water-soluble vitamins, storage of thiamin in the body is limited, so that organisms at higher trophic levels depend on a continuous intake of thiamin through their diet (Cheah et al. 2007; Combs 2012; Jurgenson et al. 2009). Moreover, thiamin levels in the worlds ocean are often very low, even below picomolar concentrations, indicating a mismatch between supply and demand where thiamin is immediately taken up by the organisms (Sañudo-Wilhelmy et al. 2014; Suffridge et al. 2018).

Not all bacteria and phytoplankton species produce thiamin and therefore some species may rely solely on an exogenous supply, so called thiamin auxotrophs (Carlucci

and Bowes 1970; Croft et al. 2006; Tang et al. 2010). Recent studies suggest that bacterial thiamin auxotrophy is more widespread and seasonally dynamic than previously suggested (Paerl et al. 2018b). Phytoplankton also show a large variation in thiamin content among phyla, indicating that the amount of thiamin available for transfer in the food web may depend on the phytoplankton species composition (Carlucci and Bowes 1972). Of the few species investigated, filamentous Cyanophyceae have been found to contain considerably higher levels of thiamin than other phytoplankton while Bacillariophyceae and Dinophyceae have an intermediate thiamin content and Cryptophyceae have lowest carbon-specific thiamin content (De Roeck-Holtzhauer et al. 1991; Fridolfsson et al. 2018; Sylvander et al. 2013). Furthermore, several different phytoplankton species are able to regulate thiamin content when exposed to abiotic stress, for instance increased/decreased temperature, salinity and light levels (Sylvander et al. 2013). Thiamin concentrations in other types of commonly occurring seston, such as heterotrophic protists and detritus are not well known.

The factors affecting thiamin concentrations in zooplankton are largely unknown. However, phytoplankton community composition can have a large effect on zooplankton ingestion and fecundity (Jónasdóttir and Kiørboe 1996; Kiørboe and Nielsen 1994; Koski and Kuosa 1999). For instance, zooplankton ingestion can be reduced by the presence of toxic or large phytoplankton that can interfere with the feeding apparatus, e.g. filamentous Cyanophyceae (Engström et al. 2000; Sopanen et al. 2006). Zooplankton can tolerate filamentous Cyanophyceae, however, their presence mostly has negative effects on zooplankton production (Engström-Öst et al. 2002; Engström et al. 2000; Hogfors et al. 2014; Motwani et al. 2018). This is related to low manageability of the filaments (Gliwicz and Siedlar 1980), production of non-ribosomal peptides, e.g. nodularin and spumigin (Mazur-Marzec et al. 2016), or inadequate fatty acid composition (Ahlgren et al. 1992).

The nutritional value of zooplanktons and their food items is often assessed by the composition and concentration of fatty acids (Ahlgren et al. 1992, 2005; Müller-Navarra and Huntley 2013). While the effect of fatty acid content on zooplankton reproductive success have received much attention (Brett et al. 2009; Jónasdóttir 1994; Jónasdóttir and Kiørboe 1996), vitamin content and transfer in the aquatic food web has been largely neglected. Therefore, the aim of this study was to investigate the seasonal variation in thiamin content of pico-, nano- and microplankton as well as in zooplankton, in the Baltic Sea and Skagerrak area. We hypothesize that the thiamin content varies seasonally, being influenced by the species-specific content and the seasonal species succession.

Materials and methods

Sampling

In the present study, we focused on copepods as they dominate (> 70%) the zooplankton community in the Baltic Sea and the Skagerrak area (Vuorinen et al. 1998; Zervoudaki et al. 2009). Samples for copepod thiamin content were collected monthly from November 2013 to December 2016. Thiamin content in pico-, nano- and microplankton of two size fractions (0.7–3 µm and > 3 µm) (commonly containing bacteria, phytoplankton, detritus and protists) were sampled from January 2015 to December 2016 in the Baltic Sea, at Linnaeus Microbial Observatory (LMO; N56°55.8540', E17°3.6420') in the western Baltic Proper, described in Legrand et al. (2015). Sampling of both copepod and the pico-, nano- and microplankton were also performed from May 2015 to February 2016 in the Skagerrak area, the Gullmar fjord (N58°15.3270', E11°27.2195'). Sampling in the field was performed between 08.00 and 10.00 local time and transported to the lab within 1 h. For logistical reasons, more samplings were performed at LMO compared to in the Gullmar fjord. Copepods were collected by oblique hauls from the top 30 m using a plankton net (Ø50 cm, 200-µm mesh size) with a fitted flowmeter (HELCOM 2017) whereas plankton of the two size fractions (0.7–3 µm and > 3 µm) were sampled by pooling water from 2, 4, 6, 8 and 10 m.

Copepods were sampled for thiamin, particulate organic carbon (POC) and particulate organic nitrogen (PON) content by picking 40 individuals per genera and sample using tweezers, in triplicates under a stereomicroscope (Olympus SZX7). Mixed community samples were collected by picking the first 50 copepod individuals encountered. Thiamin, POC and PON content in pico-, nano- and microplankton were sampled by filtering water (2 L) consecutively in two size fractions, > 3 µm (hereafter large) and 0.7–3 µm (hereafter small) (Whatman glass fiber filter, GF/D and GF/F, respectively), in duplicates. For POC and PON content, glass fiber filters were pre-combusted (475 °C, 3 h). The two size fractions provide information on thiamin content for communities dominated by phytoplankton (large), picoplankton and bacteria (small). However, detritus and heterotrophic protists can also contribute to the different size fractions. To estimate how much of the small and large size fraction that was comprised of material other than phytoplankton, total phytoplankton biomass (mgC m^{-3} , estimated from cell counts and size measurements) was subtracted from total POC content (from POC and PON element analysis) of the large size fraction and is hereafter referred to as “Other seston”. As a proportion of the bacterial community was not collected in the small

size fraction, the thiamin content in this size fraction could be underestimated. Samples for thiamin of the large and small size fractions and copepods were stored in 2 mL Eppendorf tubes and kept in -80°C until further analysis, whereas POC and PON content samples were put in H_2O_2 washed 2 mL Eppendorf tubes and immediately stored in -20°C . POC and PON filters were dried at 60°C for at least 24 h and stored in a desiccator prior to analysis with a Perkin Elmer CHNS/O Analyzer 2400 Series II. Separate samples for phytoplankton and zooplankton abundances were preserved with 2% acidic Lugol's solution and kept in the dark.

Analysis of thiamin

Thiamin was analyzed according to Pinto et al. (2002) with slight modifications according to Sylvander et al. (2013) and the limit of detection for the system used was 55 fmol and was calculated according to Suffridge et al. (2017). Briefly, thawed samples were sonicated in 1–1.5 mL 0.1 M HCl with a Vibra-Cell sonicator (amplitude 92 for filters or 40 for copepod samples, respectively) on ice for 1.5 min with 1 s pulses. Extracts were centrifuged at $16,900\times g$ at 10°C during 10 min and 700 μL of the supernatant was centrifuged once more under the same conditions. Next, 600 μL of the supernatant was mixed with 550 μL MeOH, 300 μL 1 M NaOH and 50 μL freshly made 30 mM $\text{K}_3\text{Fe}(\text{CN})_6$. Finally, the mix was filtered through a 0.45- μm PTFE/PP syringe filter. Standard solutions (1 μM) for the three types of thiamin, free thiamin (TF), thiamin monophosphate (TMP) and thiamin diphosphate (TDP) were prepared in 0.1 M HCl and aliquoted in a five-point standard series. Blank samples were prepared by mixing 600 μL 0.1 M HCl with the remaining chemicals. Standards and blanks were treated in the same way as samples, except for the sonication and centrifugation step. To ensure that the quantification was correct, a selection of samples were spiked with standards and the chromatograms inspected. Furthermore, a recovery assay was performed to assess the matrix suppression. No corrections were made since the recovery rate was $>95\%$ for all compounds investigated.

Thiamin samples were analyzed using a Hitachi Chromaster HPLC system with a Purospher[®]Star NH_2 LiChroCART[®] column (5 μm particle size, 4.6 mm [I.D.] \times 250 mm), protected by a Purospher[®]Star NH_2 LiChroCART[®] guard column (5 μm particle size, 4 mm [I.D.] \times 4 mm). Fluorescence was detected using an excitation wavelength of 375 nm and an emission wavelength of 450 nm. Injection volume was 100 μL with a flowrate of 1 mL min^{-1} . Mobile phase consisted of MeOH and 0.1 M phosphate buffer (pH 7.4) at a ratio of 43:57. Chromatograms were integrated using the software OpenLab (Agilent Technologies), and baselines were drawn automatically and inspected manually.

Three types of thiamin were analyzed, TF, TMP and TDP and these values were summed up in order to get the total thiamin content (T_{tot}).

Analysis of zooplankton, phytoplankton and bacterial abundance

Zooplankton were identified to the genus or species level under a stereomicroscope. Phytoplankton was analyzed in sedimentation chambers according to the Utermöhl method (Utermöhl 1931, 1958) and counted using an inverted microscope (Nikon TMS). Phytoplankton were identified to the genus or species level and cell measurements were used to calculate biovolume and biomass according to Olenina et al. (2006) and Edler (1979). Bacterial abundances were sampled in duplicates from the filtrate of each size fraction, as well as for non-filtered samples. Samples were preserved with formaldehyde (3.7% final concentration) and kept at -80°C until further analysis. Samples for total bacterial abundance were enumerated in a flow cytometer (Partec Cube8) with protocols adapted from Gasol and Morán (2016). Briefly, for total bacterial abundances, cells were thawed in darkness at room temperature, stained with SYBR Green for 15 min before counting (Life Technologies, 5 μM final concentration) and counted in the flow cytometer (50 μL , flow rate $1\text{ }\mu\text{L s}^{-1}$) using blue light excitation, side scatter and green fluorescent detectors. Abundance of auto fluorescent cells was analyzed without stain added to detect auto fluorescence (100 μL , flow rate $1\text{ }\mu\text{L s}^{-1}$) using blue light excitation, side scatter and red fluorescent detectors. Stained cell counts, enumerate the entire bacterial community, whereas the auto fluorescent cell counts only show the part of the bacterial community containing chlorophyll. To estimate the proportion of bacterial cells that passed the different size fractions, the bacterial abundances in each filtrate was divided by the bacterial abundance in the non-filtered sample. Bacterial abundances presented here are from the non-filtered sample.

Data handling and statistical analyses

Statistical analyses were performed using R software version 3.4.2 (R Core Team 2017). For linear mixed effects analyses (Bates et al. 2015; Pinheiro et al. 2017), analysis of variance (one-way ANOVA) and following post hoc according to Tukey (Hothorn et al. 2008), data were log transformed to conform to the assumptions of the tests. Results from linear mixed effects analyses are presented as Chi square (χ^2) values and P values in addition to subsequent post hoc tests, which are presented as z values. For linear mixed models, sampling occasion was specified as the random effects. Results from one-way ANOVA's are presented as F values and P values and the following post hoc tests are presented

as t values. Thiamin content data in the present study is presented as three measurements. Carbon-specific or nitrogen-specific thiamin content, which was calculated by dividing thiamin per sample with the carbon or nitrogen content per sample respectively, and thiamin per copepod specimen, which was calculated by dividing thiamin per sample with the number of individuals sampled. Unless stated otherwise, thiamin content refers to carbon-specific total thiamin content (T_{tot}). Thiamin ratio, here defined as thiamin content at a higher trophic level (copepods) divided with thiamin content at the basal trophic level (sum of the large and small size fraction), was calculated according to Hairston and Hairston (1993). Thiamin ratio illustrates the relationship between copepod thiamin content and the thiamin content in the large and small size fraction but does not consider the retention efficiency or cellular demands of thiamin. All graphs were created using the packages “ggplot2” (Wickham 2009) and “gridExtra” (Auguie 2017). Unless stated otherwise, the data are presented as average values and occasionally with standard deviation. Seasons are divided according to the northern hemisphere terminology, with winter (December, January, February), spring (March, April, May), summer (June, July, August) and autumn (September, October, November).

Results

Copepods

Copepod thiamin content ranged from 67 to 870 nmol g C⁻¹ (Fig. 1a) among genera and seasons with no distinct seasonal pattern, and there was no interaction effect between location and genera for copepod thiamin content (Chi square test, $\chi^2 = 4.95$, $P = 0.18$). Hence, concentrations of thiamin among genera displayed the same general patterns in both locations. However, there were differences in thiamin content between genera at both LMO (Linnaeus Microbial Observatory, Baltic Sea) and the Gullmar fjord (Skagerrak area) (ANOVA, $F(3,183) = 15.76$, $P < 0.001$; $F(3,41) = 18.63$, $P < 0.001$, respectively) (Fig. 1b). At LMO, *Acartia* sp. had a higher thiamin content compared to both *Temora* sp. and *Pseudocalanus* sp. (Tukey HSD, $t = 3.78$, $P < 0.01$; $t = 6.33$, $P < 0.001$, respectively) during the whole study period. Furthermore, *Pseudocalanus* sp. had lower thiamin content than *Temora* sp. and the Mixed community (Tukey HSD, $t = -3.87$, $P < 0.001$; $t = -5.29$, $P < 0.001$, respectively). In the Gullmar fjord, *Acartia* sp. had higher thiamin content than *Temora* sp., *Pseudocalanus* sp. and the Mixed community (Tukey HSD, $t = 5.67$, $P < 0.001$; $t = 6.25$, $P < 0.001$; $t = 3.56$, $P < 0.01$, respectively). When comparing thiamin content between genera at the two locations, the content was higher for the Mixed community from LMO compared to the Gullmar fjord (ANOVA, $F(1,41) = 9.48$,

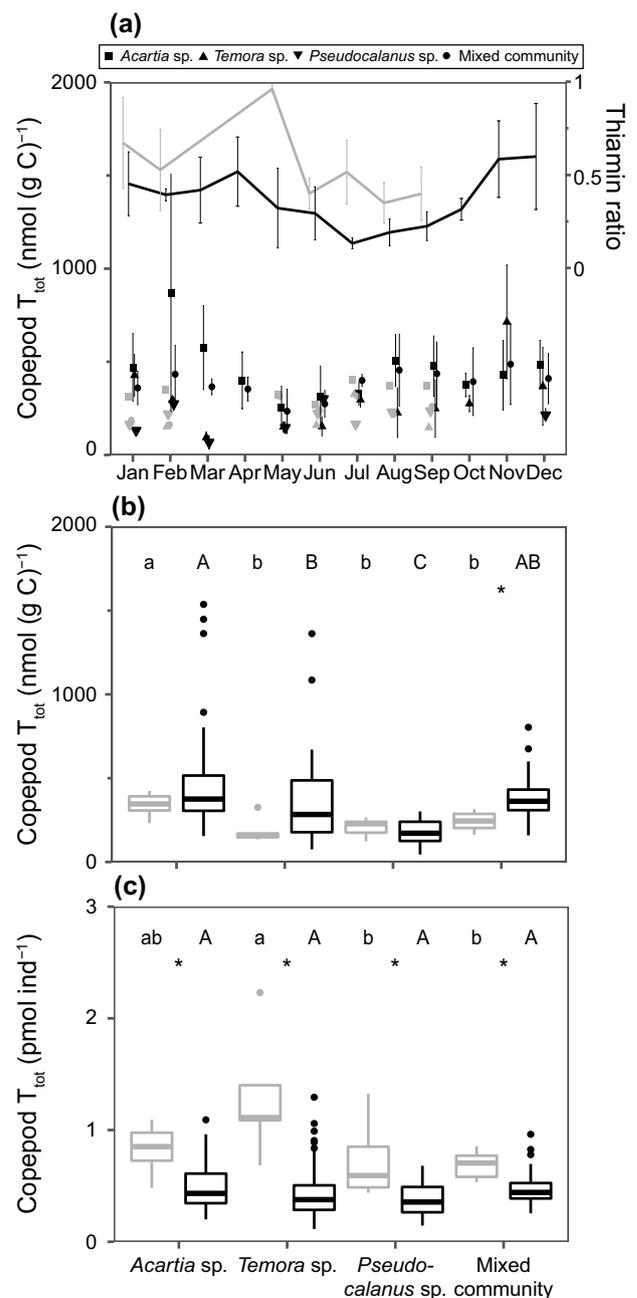


Fig. 1 Copepod thiamin content at LMO (Baltic Sea, black) and the Gullmar fjord (Skagerrak area, grey). **a** Average seasonal thiamin content in different copepod genera (left y-axis). Thiamin ratio (thiamin content in copepods divided by the large and small plankton size fraction) for the two locations is presented in the top part of the plot (right y-axis). **b** Boxplot for carbon-specific thiamin content in different copepod genera. **c** Boxplot for thiamin content per copepod specimen in different copepod genera. Error bars illustrate standard deviation for inter-annual variation for copepod thiamin content, and standard deviation for different copepod genera for thiamin ratio. Boxplots show median, interquartile ranges (box), ranges (vertical lines) and outliers (points). Different letters indicate significant differences, capital letters for LMO, and small letters for the Gullmar fjord. Significant differences between congeners from different locations are indicated with *

$P < 0.01$), whereas thiamin content in *Acartia* sp., *Temora* sp. and *Pseudocalanus* sp. was similar between locations (ANOVA, $F(1,101) = 1.98$, $P = 0.16$; $F(1,56) = 2.39$, $P = 0.13$; $F(1,26) = 2.86$, $P = 0.10$, respectively) (Fig. 1b).

In contrast, when assessing thiamin content per copepod specimen, the results differed somewhat and there was an interaction effect between location and species (Chi square test, $\chi^2 = 8.18$, $P < 0.05$). Hence, when comparing thiamin per copepod specimen between locations, *Acartia* sp., *Temora* sp. and *Pseudocalanus* sp. in the Skagerrak had higher levels of thiamin compared to congeners from the Baltic Sea area (Tukey HSD, $z = 4.30$, $P < 0.001$; $z = 5.90$, $P < 0.001$; $z = 3.49$, $P < 0.01$, respectively), (Fig. 1c). At LMO there were no differences in the levels of thiamin per copepod specimen among different genera, whilst in the Gullmar fjord, *Temora* sp. had higher levels of thiamin per copepod specimen than both *Pseudocalanus* sp. and the Mixed community (Tukey HSD, $z = 3.83$, $P < 0.01$; $z = 3.12$, $P < 0.05$, respectively), but there was no difference between *Temora* sp. and *Acartia* sp. (Tukey HSD, $z = 2.21$, $P = 0.30$), (Fig. 1c). For both locations, thiamin content in copepods was negatively related to copepod size, where larger individuals showed a lower thiamin content than smaller individuals (Fig. 2).

There was no overall interaction effect between location and genera for nitrogen-specific thiamin content in copepods (Chi square test, $\chi^2 = 3.36$, $P = 0.34$). Hence, nitrogen-specific concentrations of thiamin among genera displayed the same general patterns in both locations with the only exception of the Mixed community, having higher levels at LMO compared to the Gullmar fjord (ANOVA, $F(1,41) = 11.19$, $P < 0.01$) (Fig. S1a). There were also differences between genera within LMO and the Gullmar fjord (ANOVA, F

(3,183) = 10.46, $P < 0.001$; $F(3,41) = 15.16$, $P < 0.001$, respectively) (Fig. S1a). The results resembled those for carbon-specific thiamin content, with *Acartia* sp. having a higher nitrogen-specific thiamin content compared to both *Temora* sp. and *Pseudocalanus* sp. (Tukey HSD, $t = 3.62$, $P < 0.01$; $t = 4.86$, $P < 0.001$, respectively) during the whole study period at LMO. In the Gullmar fjord, *Acartia* sp. had higher nitrogen-specific thiamin content than *Temora* sp., *Pseudocalanus* sp. and the Mixed community (Tukey HSD, $t = 5.8$, $P < 0.001$; $t = 4.98$, $P < 0.001$; $t = 2.99$, $P < 0.05$, respectively).

Thiamin in copepods was mostly present in the unphosphorylated type, TF, and thiamin monophosphate, TMP, at both LMO and the Gullmar fjord (Fig. S2a, b). The proportion of the active type thiamin diphosphate, TDP, was higher during the spring compared to the rest of the year at LMO (ANOVA, $F(3,183) = 3.07$, $P < 0.05$), whereas in the Gullmar fjord the proportion of TDP did not change over the year (ANOVA, $F(3,41) = 1.14$, $P = 0.34$).

Copepod abundance showed seasonal variation with *Acartia* sp. and *Temora longicornis* normally constituting $78 \pm 18\%$ of the total copepod abundance in the Baltic Sea whereas *Pseudocalanus* sp. was less abundant ($8 \pm 7\%$) (Fig. S3a). In the Skagerrak, the dominant groups were slightly different, with both *Acartia* sp. and *Pseudocalanus* sp. dominating during spring and summer and *Oithona* sp. was present throughout the study period (Fig. S3b). Copepods were larger (prosoma length) (ANOVA, $F(1,913) = 28.54$, $P < 0.001$) and had higher POC (ANOVA, $F(1,120) = 81.28$, $P < 0.001$) and PON content (ANOVA, $F(1,120) = 92.45$, $P < 0.001$) in the Gullmar fjord compared to congeners from LMO in the Baltic Sea (Table 1). C:N ratio did not differ between the two locations (ANOVA, $F(1,120) = 0.33$, $P = 0.56$) (Table 1).

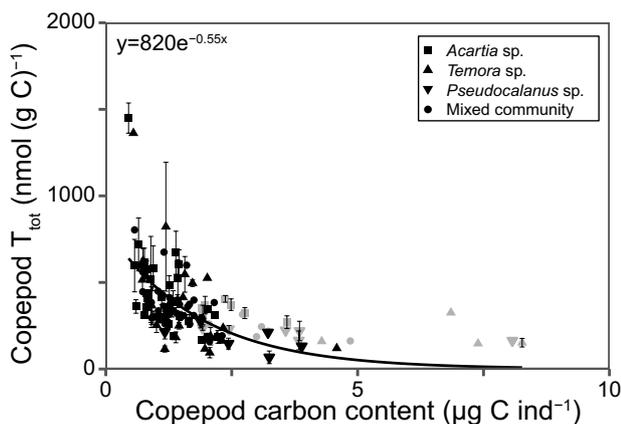


Fig. 2 Correlation between carbon-specific copepod thiamin content and copepod carbon content in different copepod genera at LMO (Baltic Sea, black) and the Gullmar fjord (Skagerrak area, grey). Error bars illustrate standard deviation between replicates. Equation for exponential regression is presented in top left corner

Thiamin in pico-, nano- and micro-plankton

Thiamin content in pico-, nano- and micro-plankton sampled in a small ($0.7\text{--}3\ \mu\text{m}$) and a large size fraction ($> 3\ \mu\text{m}$) showed seasonal variation, being highest in July, August and September (Fig. 3a). Thiamin content in the large size fraction ranged from 123 to $1384\ \text{nmol g C}^{-1}$ at LMO and from 41 to $188\ \text{nmol g C}^{-1}$ in Gullmar fjord. The small size fraction ranged from 425 to $1750\ \text{nmol g C}^{-1}$ at LMO and from 288 to $676\ \text{nmol g C}^{-1}$ in the Gullmar fjord. Hence, thiamin content in the large size fraction varied by approximately a factor ten at LMO throughout the sampling whereas it only varied twofold in the Gullmar fjord (Fig. 3a, c). Hence, there was an interaction effect between location and size fraction for thiamin content (Chi square test, $\chi^2 = 18.94$, $P < 0.001$).

Furthermore, the small size fraction had a higher thiamin content than the large size fraction both at LMO (Tukey HSD, $z = 14.07$, $P < 0.001$) and the Gullmar fjord

Table 1 Average size (prosome length), carbon (POC) and nitrogen (PON) content and C:N ratios in different copepod genera at LMO (Baltic Sea) and the Gullmar fjord (Skagerrak area)

Location	Genera	<i>n</i> PL	Prosome length (μm)	<i>n</i> POC/PON	POC (μg ind ⁻¹)	PON (μg ind ⁻¹)	C:N
LMO	<i>Acartia</i> sp.	294	604 (± 104)	36	1.2 (± 0.5)	0.29 (± 0.12)	4.3 (± 0.8)
LMO	<i>Temora</i> sp.	159	480 (± 79)	32	1.6 (± 0.8)	0.37 (± 0.15)	4.4 (± 0.7)
LMO	<i>Pseudocalanus</i> sp.	50	644 (± 101)	9	2.4 (± 1.1)	0.45 (± 0.19)	5.4 (± 1.0)
LMO	Mixed community	716	549 (± 113)	35	1.3 (± 0.4)	0.31 (± 0.09)	4.3 (± 0.6)
Gullmar fjord	<i>Acartia</i> sp.	47	925 (± 421)	7	2.4 (± 0.6)	0.59 (± 0.10)	4.1 (± 0.4)
Gullmar fjord	<i>Temora</i> sp.	34	609 (± 194)	4	6.7 (± 1.7)	1.68 (± 0.53)	4.1 (± 0.4)
Gullmar fjord	<i>Pseudocalanus</i> sp.	45	718 (± 127)	6	3.9 (± 2.2)	0.87 (± 0.53)	4.6 (± 0.6)
Gullmar fjord	Mixed community	225	702 (± 288)	7	3.0 (± 1.0)	0.70 (± 0.23)	4.3 (± 0.5)

Values in parentheses are standard deviation and *n* shows number of samples that average values are based on

(Tukey HSD, $z = 17.79$, $P < 0.001$) (Fig. 3b). Thiamin content in the large size fraction was higher in the Baltic Sea area compared to the Skagerrak area (Tukey HSD, $z = 6.16$, $P < 0.001$), and marginally higher in the small size fraction (Tukey HSD, $z = 2.46$, $P = 0.051$) (Fig. 3b). Additionally, thiamin content in the two size fractions was strongly correlated (Pearson correlation, $r = 0.87$, $P < 0.001$, $N = 58$) (Fig. 3c).

Nitrogen-specific thiamin content in the two size fractions of plankton displayed a similar seasonal variation as carbon-specific thiamin content even if the absolute values were higher, due to an average C:N ratio around seven (Fig. S1b). There was an interaction effect between location and size fraction for nitrogen-specific thiamin content (Chi square test, $\chi^2 = 10.52$, $P < 0.001$). Nitrogen-specific thiamin content was higher at LMO compared to the Gullmar fjord in the small (Tukey HSD, $z = 2.86$, $P < 0.05$) and large size fraction (Tukey HSD, $z = 6.29$, $P < 0.001$). Moreover, the levels were higher in the small size fraction at LMO (Tukey HSD, $z = 9.36$, $P < 0.001$) and the Gullmar fjord (Tukey HSD, $z = 12.46$, $P < 0.001$), compared to the large size fraction.

Thiamin in the two size fractions of plankton was mostly present in the unphosphorylated type, TF, at both LMO and the Gullmar fjord (Fig. S2c–f). The proportion of the active type, TDP, was lower in the small size fraction, during the spring and summer months compared to the autumn and winter months at LMO (ANOVA, $F(3,40) = 19.48$, $P < 0.001$), whereas in the Gullmar fjord the proportion of TDP was similar over all seasons (ANOVA, $F(3,10) = 2.2$, $P = 0.15$) (Fig. S2c–d). TDP was lowest during summer at LMO in the large size fraction (ANOVA, $F(3,40) = 4.41$, $P < 0.01$), but no seasonal differences in the Gullmar fjord were observed (ANOVA, $F(3,10) = 1.84$, $P = 0.19$) (Fig. S2e–f). Furthermore, the proportion of TDP was similar between the two locations in both the small (ANOVA, $F(1,56) = 2.21$, $P = 0.14$) and the large size fraction (ANOVA, $F(1,56) = 0.47$, $P = 0.5$).

Phytoplankton biomass varied seasonally with changes among the dominating groups (Fig. 4a, b). Dinophyceae was present throughout the 2 years and dominated during spring. During summer, a large part of the phytoplankton community at LMO consisted of Cyanophyceae, whereas in the Gullmar fjord no Cyanophyceae was present during the study period. Prymnesiophyceae contributed during 2016 to more of the biomass of the phytoplankton community than in 2015. The amount of Other seston also varied seasonally (Fig. 4a, b), being higher during the productive period from spring to autumn and lower during winter, throughout the 2 years of sampling. The levels as well as the trend was similar at LMO and in the Gullmar fjord.

Bacteria also showed seasonal variation, peaking during early autumn and having their lowest abundance in winter, total bacterial abundance ranging from 2.8×10^5 to 1.8×10^6 cells mL⁻¹, whereas the auto fluorescent cell abundance ranged from 3.3×10^3 to 4.7×10^4 cells mL⁻¹, at LMO (Fig. S4a). In the Gullmar fjord, total bacterial abundance ranged from 4.6×10^4 cells mL⁻¹ during winter to 2.1×10^6 cells mL⁻¹ during late spring whereas auto fluorescent cell abundance ranged from 6×10^2 to 4.7×10^4 cells mL⁻¹ (Fig. S4b). The two locations did not differ in the amount of POC (ANOVA, $F(1,114) = 0.019$, $P = 0.89$), PON (ANOVA, $F(1,114) = 0.37$, $P = 0.54$) or the ratio between them (C:N; ANOVA, $F(1,114) = 1.89$, $P = 0.17$) for either of the two size fractions (Table S1). The majority of the bacteria passed through the first filter (approximately 85%) and hence did not largely contribute to thiamin content in the large size-fraction. However, approximately 66% of the bacteria were caught on the final filter and thereby largely contributed to the thiamin content in the small size-fraction.

Thiamin in different trophic levels

Thiamin content in copepods was correlated with thiamin content in both the large and the small size fraction of

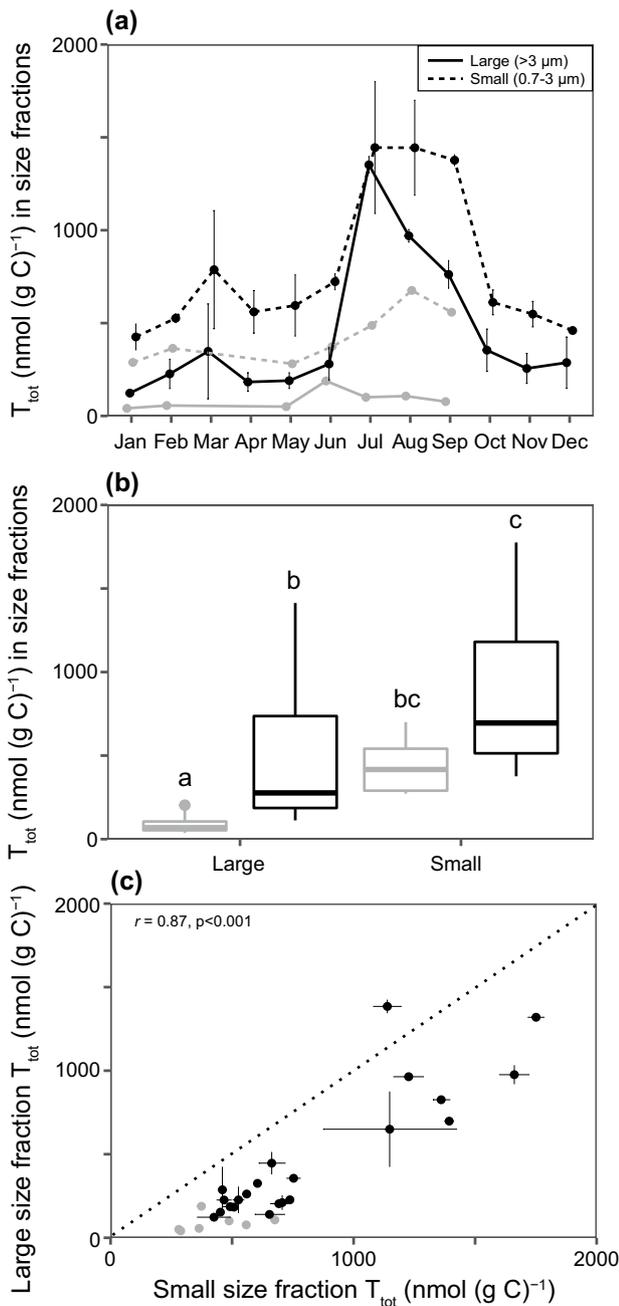


Fig. 3 Thiamin content in the two size fractions at LMO (Baltic Sea, black) and the Gullmar fjord (Skagerrak area, grey). **a** Average seasonal thiamin content in the two size fractions. **b** Boxplot for thiamin content in size fractions and locations. **c** Correlation for thiamin content in the two size fractions. Size fractions are indicated by solid (large, > 3 μm) and dashed (small, 0.7–3 μm) lines. Error bars illustrate standard deviation for inter-annual variation in **a**, and standard deviation of technical duplicates in **c**. Boxplots show median, inter-quartile ranges (box), ranges (vertical lines) and outliers (points). Different letters indicate significant differences. Dotted line illustrate hypothetical 1:1 relationship

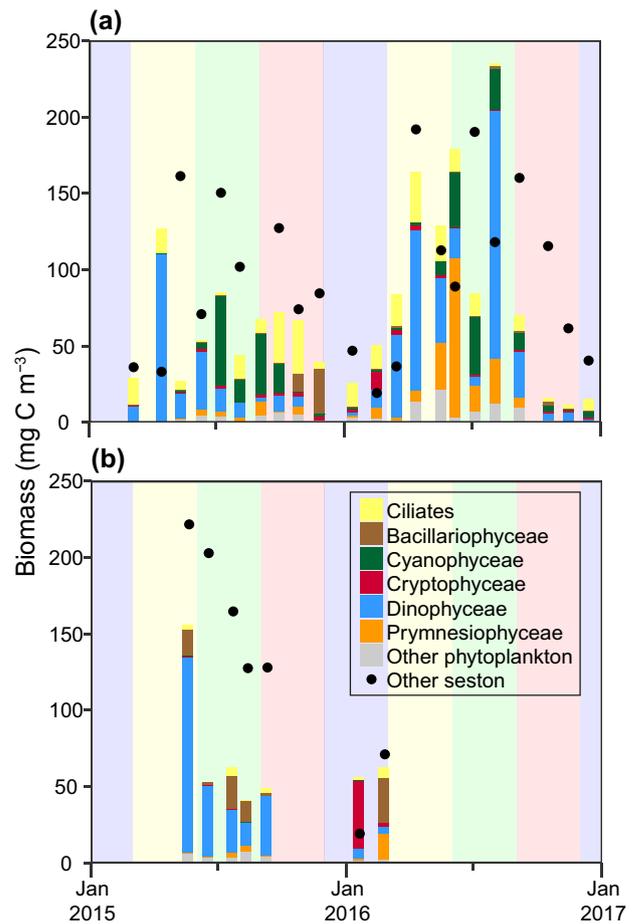


Fig. 4 Phytoplankton community composition and biomass (bars) and Other seston biomass (black circles; i.e. non-phytoplankton seston, typically detritus, protists etc.) at LMO (Baltic Sea; **a**) and in the Gullmar fjord (Skagerrak area; **b**). Background color illustrate season, winter (Dec, Jan, Feb; blue), spring (Mar, Apr, May; yellow), summer (Jun, Jul, Aug; green) and autumn (Sep, Oct, Nov; red)

plankton (Pearson correlation, $r=0.31$, $P < 0.001$, $N=154$; $r=0.35$, $P < 0.001$, $N=154$, respectively) (Fig. 5a, b). However, when this relationship was investigated during periods of high versus low thiamin content in the two size fractions (high was defined as being higher than half-maximum thiamin content, $> 676 \text{ nmol g C}^{-1}$), this relationship changed. When thiamin content was relatively low in the large size fraction, there was a positive correlation (Pearson correlation, $r=0.26$, $P < 0.001$, $N=118$) whereas when the thiamin content was higher, there was a negative correlation (Pearson correlation, $r=-0.34$, $P < 0.001$, $N=36$).

Thiamin ratio between the large and small size fraction and copepods was lower during the summer months compared to the rest of the year (Fig. 1a), as a result of an increased thiamin content in the pico-, nano- and microplankton while the thiamin content in the copepods did not change to the same extent. Furthermore, thiamin ratio was

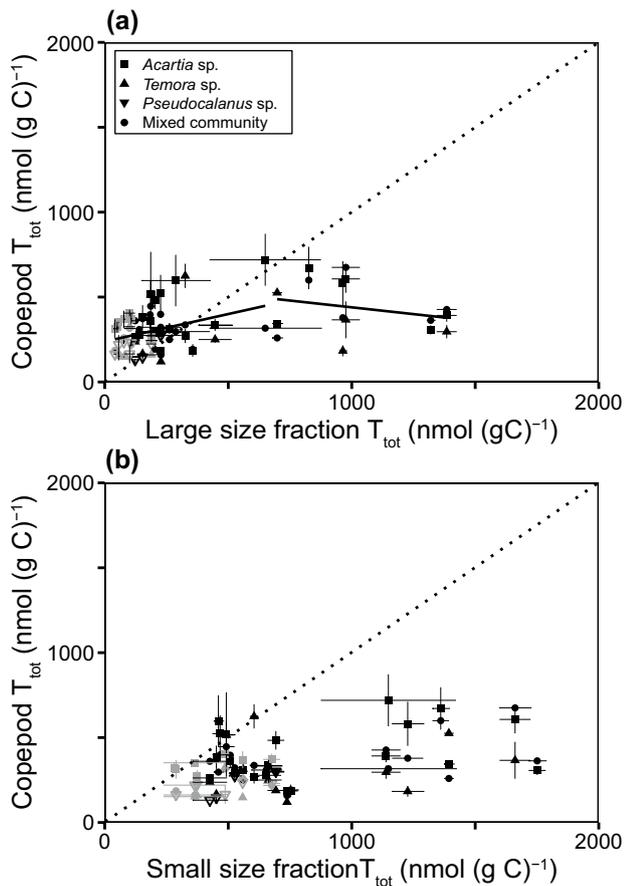


Fig. 5 Relationship between thiamin content in the two size fractions and copepods thiamin content at LMO (Baltic Sea, black) and the Gullmar fjord (Skagerrak area, grey). **a** Correlation between copepod thiamin content and thiamin content in the large size-fraction ($> 3 \mu\text{m}$). **b** Correlation between copepod thiamin content and thiamin content in the small size-fraction ($0.7\text{--}3 \mu\text{m}$). Error bars illustrate standard deviation between replicates. Regression lines in **a** are for periods of high and low thiamin content. Dotted line illustrate hypothetical 1:1 relationship

higher in the Gullmar fjord compared to LMO in the Baltic Sea throughout the study (ANOVA, $F(1,80) = 12.62$, $P < 0.01$). Maximum thiamin ratio at LMO was found in November (0.58) and December (0.6), whereas in the Gullmar fjord the maximum was found in May with 0.96. Lowest thiamin ratio at LMO during July (0.13) and August (0.19). Also in the Gullmar fjord, the minimum thiamin ratio (0.34) in August.

Discussion

Zooplankton thiamin dynamics in aquatic systems has not been thoroughly described before. Here we show that there was only a small seasonal variation in copepod thiamin content. However, thiamin content differed between copepod

genera within locations. For example, during the entire study period, *Acartia* sp. showed the highest carbon-specific thiamin content of all copepod species at both LMO (Baltic Sea) and the Gullmar fjord (Skagerrak area). Furthermore, thiamin content per copepod specimen did not differ among Baltic Sea genera, whereas copepods from the Gullmar fjord showed inter-specific differences, with *Temora* sp. having the highest thiamin content per copepod specimen. For all investigated species, the thiamin content per copepod specimen was generally higher in the Gullmar fjord compared to congeners from LMO.

Additionally, thiamin content had a negative relationship with copepod size (carbon content), a type of relationship which has also been found in blue mussels (*Mytilus* sp.) (Balk et al. 2016). Hence, highest thiamin content was found in the smaller part of the copepod community and, reversely, larger individuals had the lowest carbon-specific content. This is contrary to, for instance protein or fatty acid content, as larger copepod individuals tend to have a higher carbon-specific content of these compounds than smaller individuals (Helland et al. 2003). Overall, our findings of higher thiamin content in smaller copepods together with the reported change in copepod community composition, with decreasing abundances of *Pseudocalanus* sp. as *Acartia* sp. has increased (Möllmann et al. 2008), point towards an increase of smaller-bodied zooplankton with a high thiamin content in the Baltic Sea. The main planktivorous fish species in the system, sprat (*Sprattus sprattus*) and herring (*Clupea harengus*) feed selectively on larger copepod species, e.g. *Pseudocalanus*, over smaller, like *Acartia* sp. (Casini et al. 2004). The seasonal succession of the dominant zooplankton and phytoplankton groups in our study was similar to previous findings in this area (Diaz-Gil et al. 2014; Wasmund et al. 2011). Zooplankton community composition, with a high prevalence of *Acartia* sp., *T. longicornis* and *Pseudocalanus* sp. as well as higher abundances of zooplankton during summer and autumn, is consistent with previous studies (Diaz-Gil et al. 2014). Moreover, the phytoplankton community during spring is often dominated by Dinophyceae and during the summer Cyanophyceae dominate, whereas Bacillariophyceae or Dinophyceae often peak during autumn (Wasmund et al. 2011). Prymnesiophyceae can be present in large numbers episodically (Majaneva et al. 2012), and in our study, Prymnesiophyceae abundance was elevated the second year.

Thiamin content in two size fractions of plankton (containing bacteria, phytoplankton, detritus and protists) varied over the seasons with higher levels during summer with the small size fraction having an overall higher thiamin content. In this study, about one-third of the free living bacterial community were generally smaller than $0.7 \mu\text{m}$ and were not caught on any of the filters, suggesting that picoplankton and bacteria could contribute even more to the total amount

of thiamin in the aquatic food web than suggested in the present study. Many picoplankton and bacteria are believed to be able to synthesize thiamin de novo and a large fraction out of over 400 surveyed marine bacteria genomes had the appropriate genes for synthesis (Sañudo-Wilhelmy et al. 2014). However, recent studies suggest that the level of auxotrophy in natural bacterial communities may be much higher and variable than previously found which could suggest a dynamic seasonal succession in thiamin producing and auxotrophic bacteria (Paerl et al. 2018b). Members of Cyanophyceae generally have the genes necessary to produce thiamin and a majority of these small, unicellular species e.g. *Synechococcus* sp. and *Prochlorococcus* sp. (Sañudo-Wilhelmy et al. 2014), would be collected in the small size fraction used in the present study. Picoplankton has been suggested to have high thiamin cell⁻¹ quota (Paerl et al. 2015), which is in agreement with our findings. One reason for the higher thiamin content in the small compared to the large size-fraction could be that uptake rates of thiamin are higher for picoplankton and bacteria than for larger phytoplankton communities (Koch et al. 2012). This can have implications for the community composition of bacteria, phytoplankton and heterotrophic protists since thiamin auxotrophs could be negatively affected if thiamin is depleted (Kraft and Angert 2017). Our results further support that in addition to being a vitamin source, picoplankton and bacteria could also be considered as a sink of vitamins, if they outcompete other phytoplankton in terms of thiamin uptake but in turn are not consumed by zooplankton to the same extent due to their small size (Koch et al. 2011). Furthermore, in addition to having higher uptake rates for thiamin, picoplankton also have a higher demand than larger phytoplankton (Koch et al. 2012), which is consistent with the thiamin content in the two size fractions in the present study. Gomez-Consarnau et al. (2018) recently found that even if the thiamin dependent transcripts of the investigated picoplankton community (0.2–3 µm) was weakly expressed, there was a positive relationship between ambient thiamin concentrations and chlorophyll *a* levels. Whether larger phytoplankton could be a source of thiamin to the picoplankton community by excretion, lysis or other mechanisms, still needs further investigations.

Furthermore, thiamin content in the two size fractions of plankton was higher at LMO compared to the Gullmar fjord, especially during summer when also Cyanophyceae, Prymnesiophyceae and Other seston were major contributors to the total biomass of pico-, nano- and micro-plankton. This is in agreement with previous studies where Cyanophyceae was found to have relative high thiamin content compared to other phytoplankton (Fridolfsson et al. 2018; Sylvander et al. 2013). It is not fully established if filamentous Cyanophyceae are able to produce thiamin de novo (Sañudo-Wilhelmy et al. 2014),

but thiamin auxotrophy in Cyanophyceae has to our knowledge not been reported. Cyanophyceae can also produce cobalamin (vitamin B₁₂), but recently it was found that many Cyanophyceae actually produce a less bio-available form, pseudo-cobalamin, which shows that there may be a discrepancy in the occurrence of genes and actual production of vitamins (Heal et al. 2017; Helliwell et al. 2016; Walworth et al. 2018). This shows that more information is needed about the vitamin production and content in different phytoplankton to increase our understanding on the vitamin dynamics in the aquatic food web. Several recent papers have provided new knowledge on thiamin synthesis and alternative precursors. Traditionally, thiamin was thought to be synthesized by the coupling of a thiazole (HET) and a pyrimidine (HMP) moiety (e.g. Kraft and Angert 2017). Recently, it was reported that some marine picoeukaryotic phytoplankton species can grow efficiently on a thiazole-related precursor, carboxythiazole (cHET), at lower levels than reported for thiamin (Paerl et al. 2018a). The ubiquitous bacteria SAR11 has been found to be auxotrophic for the HMP moiety and cannot use exogenous thiamin to support growth (Carini et al. 2014). Some Haptophyceae have also been reported to grow more efficiently on HMP and the salvage-related analog AmMP precursors than thiamin when supplied at the similar concentrations (Gutowska et al. 2017). Altogether, this demonstrates that thiamin dynamics, synthesis and transfer is not fully understood and should be further investigated.

Thiamin content in the two size fractions of pico-, nano- and micro-plankton varied as much as tenfold at LMO but only twofold in the Gullmar fjord during the study period. The large seasonal variation and the difference between locations could potentially be attributed to large filamentous Cyanophyceae which were almost absent in the Skagerrak area (Gullmar fjord) but very abundant during late summer to early autumn in the Baltic Sea, at LMO. Additionally, not only bacteria and phytoplankton were sampled, but detritus and protists could potentially contribute to the thiamin content in the size-fractionated samples. Detritus concentrations in seston are variable and can constitute a major fraction of POC (Andersson and Rudehäll 1993). Storage of thiamin is limited and the turnover is generally high (Combs 2012; Jurgenson et al. 2009), suggesting that the concentrations of thiamin in detritus is relatively low although there are no studies confirming this conclusion. Finally, seasonal differences in thiamin concentrations could also be attributed to within-species differences in thiamin concentration. For example, when supplied thiamin at enriched levels or naturally occurring levels, total thiamin content of the haptophyte *Emiliania huxleyi* was found to vary tenfold (Gutowska et al. 2017). At enriched levels, *E. huxleyi* reached thiamin content similar to spring levels in the large size fraction from LMO, while at

naturally occurring levels, thiamin content was in the range of the lower levels observed in the Gullmar fjord.

In the two size fractions of plankton, the unphosphorylated type, TF, was the dominating type of thiamin. Copepods had slightly higher proportions of the neutral type, TMP, and the active type, TDP, even if TF and TMP represented the majority of the thiamin found in copepods. In animal tissue, thiamin is usually present in the active type as TDP but in plant-based food, thiamin mainly occurs as TF (Combs 2012). In larger organisms, e.g. blue mussels and eiders (*Somateria mollissima*), TDP is the main type of thiamin (Balk et al. 2016; Mörner et al. 2017), however, sprat and herring have been found to have higher relative proportions of TF (Vuorinen et al. 2002). For thiamin to be taken up, it is first dephosphorylated to TF and then rephosphorylated intracellularly to TMP and TDP (Manzetti et al. 2014). Hence, when assessing dietary uptake and transfer of thiamin, the level of phosphorylation may not be a relevant factor.

Thiamin content in copepods correlated with thiamin content in the two size fractions of pico- nano- and micro-plankton, however, this relationship was considerably lower than the 1:1 relationship. Interestingly, during periods of lower thiamin content, copepod thiamin was positively related to thiamin content in the two size fractions, whereas during periods of high thiamin content in plankton, this relationship was negative. This suggests that a part of the thiamin is not available for higher trophic levels. Many copepods feed selectively, mostly defined by prey size, and have an optimal predator:prey ratio around 0.1 (Hansen et al. 1994). Copepods in the present study had an average prosome length of 600 μm (Baltic Sea) and 700 μm (Skagerrak). The optimal prey size would, therefore, be around 60 and 70 μm , in the two systems. During periods of relatively higher thiamin content in the two size fractions of plankton, the proportion of large phytoplankton species was also elevated, e.g. filamentous Cyanophyceae contributed to one-third of the phytoplankton community biomass, with levels of Prymnesiophyceae and Other seston also being elevated. Several Prymnesiophyceae are toxic (Johansson and Granéli 1999a, b) and lower filtration rates, ingestion and reproduction has been shown for different copepod species when exposed to these phytoplankton (Sopanen et al. 2006). Filamentous Cyanophyceae are proposed to be difficult for copepods to ingest as the filaments can be a couple of hundred micrometers long (Schmidt et al. 2002), and can form large bundles (Gliwicz and Siedlar 1980), but can also be toxic (Mazur-Marzec et al. 2016) and have low nutritional value (Ahlgren et al. 1992). An additional factor that may affect the correlation between thiamin in zooplankton and their food source is the potential presence of thiamin producing gut microbiota. There are examples of exchange of vitamin B₁₂ between members of the microbial community (Helliwell et al. 2016;

Walworth et al. 2018) but this has, to our knowledge, not been described for zooplankton and their gut microbiome.

Average thiamin ratio in the present study is in the same range as carbon transfer efficiency reported for the Baltic Proper (0.006–0.7, Dahlgren et al. 2010), however, the thiamin ratio was higher during winter and spring than in summer, in contrast to carbon transfer efficiency. Moreover, throughout the study, the thiamin ratio was higher in the Gullmar fjord compared to LMO and one of the major differences between the two systems was that Prymnesiophyceae was only present in low numbers and Cyanophyceae was close to absent in the Skagerrak area (Gullmar fjord). Presence of Prymnesiophyceae and filamentous Cyanophyceae during summer in the Baltic Sea can hence be one reason why the thiamin ratio in this system is lowest during summer and consistently lower than in the Gullmar fjord. Filamentous Cyanophyceae have recently been found to contain high concentrations of thiamin but this is not transferred to copepods during feeding experiments (Fridolfsson et al. 2018), giving a lower thiamin ratio. Still, for more exact thiamin transfer estimates, future studies should include retention efficiency and cellular demands of thiamin.

Food quality is often defined by fatty acid content and composition (Ahlgren et al. 1992; Müller-Navarra and Huntley 2013). Reduced levels of specific fatty acids have been shown to not only negatively affect zooplankton growth rates and fecundity, but also affecting the transfer of carbon in the food web (Müller-Navarra et al. 2000). Furthermore, zooplankton fatty acid content is known to be highly dependent on phytoplankton fatty acid content and composition, but zooplankton also shows large inter-specific differences (Brett and Müller-Navarra 1997; Tiselius et al. 2012). Similarly, we show that copepod thiamin content is related to the thiamin content in pico-, nano- and micro-plankton and that there are differences in thiamin content between copepod species. Hence, our study indicates that processes at the base of the aquatic food web can be related to thiamin concentrations in higher trophic levels.

Large-scale alterations have occurred in multiple trophic levels in the Baltic Sea during the past 4 decades, including a shift in dominance from Bacillariophyceae to Dinophyceae (Wasmund 2017) and increases in frequency and size of filamentous Cyanophyceae blooms during the summer (Wasmund et al. 2011). Also the zooplankton and fish communities in the Baltic Sea have changed, with dominance shifting from *Pseudocalanus* sp. to *Acartia* sp., whereas sprat and herring have increased in abundance at the expense of the piscivorous cod (*Gadus morhua*) (Casini et al. 2008; Möllmann et al. 2008). Deficiency of thiamin has been reported to negatively affect populations of birds and fish, from different ecosystems, including the North American Great Lakes, the New York Finger Lakes and the Baltic Sea (Balk et al. 2009; Bengtsson et al. 1999;

Fisher et al. 1995; Fitzsimons et al. 1999). Thiamin deficiency in the Baltic Sea occurs episodically, causing elevated mortality in salmonid offspring as well as paralysis and mass death in sea birds (Balk et al. 2009; Bengtsson et al. 1999). Recent studies also indicate that several species, including mussels, fish and sea birds, may suffer from sub-lethal thiamin deficiency (Balk et al. 2016; Mörner et al. 2017). Thiamin deficiency in salmon (*Salmo salar*) in the Baltic Sea has been found to be related to high abundances of sprat (Mikkonen et al. 2011) and a low thiamin per unit energy levels in the salmon's prey, sprat and herring (Keinänen et al. 2012, 2017). However, the cause of low thiamin levels in many other organisms remains unclear and increased understanding of the dynamics of thiamin in the aquatic food web is necessary.

In conclusion, we report a lower thiamin ratio (see Fig. 1) in the Baltic Sea compared to the Skagerrak area. The lower thiamin ratio mainly occurred during summer when abundances of filamentous Cyanophyceae, Prymnesiophyceae and Other seston (detritus, protists etc.) were high. Large filamentous Cyanophyceae have been shown to have a high thiamin content not being transferred to zooplankton due to limited edibility (Fridolfsson et al. 2018). Copepods feed optimally on a specific size range, which is related to the size of copepods, and if the phytoplankton is much larger or smaller, the copepod cannot ingest it. The small size fraction of plankton had higher thiamin content than the large size fraction, suggesting a vital role of bacteria and picoplankton as a source of essential thiamin to larger phytoplankton, protists and zooplankton in the aquatic food web. Our study highlights the need to consider not only the fatty acids but also edibility and vitamins when discussing zooplankton food quality and this article adds to our understanding of the production and transfer of this essential micronutrient in the base of the ecosystem.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and animal rights statement This article does not contain any studies with human participants, whilst sampling, care and experimental use of animals followed national guidelines.

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