The contribution of zooplankton to methane supersaturation in the oxygenated upper waters of the central Baltic Sea

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Abstract

We report on methane enrichments that were observed during summer in the upper water column of the Gotland Basin, central Baltic Sea. In the eastern part of the basin, methane concentrations just below the thermocline varied between 15 nM and 77 nM, in contrast to the western part where no methane enrichments could be detected. Stable carbon isotope ratios of methane (δ13C-CH4 of -67.6‰) indicated its in situ biogenic origin from CO2 reduction, which was supported by clonal sequences that clustered with Methanomicrobiaceae, a family of methanogenic Archaea. Incubation experiments with a Temora longicornis dominated seston fraction obtained from the relevant depth showed a positive correlation between seston concentration and methane production rates. Our results, in combination with previous literature outcomes, suggest that the methane enrichment in the eastern basin might be sustained by a diet-consumer relationship between the dinoflagellate Dinophysis norvegica and the copepod T. longicornis. However, our mass balance indicates that a local methane production of 110 pmol L⁻¹ d⁻¹ was needed to maintain the methane enrichment, and that the estimated production rate from our incubation experiments of 0.3 pmol CH4 d⁻¹ per adult T. longicornis (about 1 pmol L⁻¹ d⁻¹) was too low to maintain the methane enrichment by zooplankton associated methane production only. These calculations also showed that methane was consumed below the thermocline and not transported into the upper-ocean, suggesting that other sources in the mixed layer in the range of 95 pmol L⁻¹ d⁻¹ are needed to maintain the observed methane air–sea flux.

Methane (CH₄) is an important atmospheric trace gas which directly and indirectly influences the global climate (IPCC 2013). While the total global emissions of methane into the atmosphere are constrained reasonably well, poorly quantified emissions of the individual sources lead to weak source-strength estimates, which vary by a factor of two between different studies (Dlugokencky et al. 2011). Despite the fact that the world's oceans cover 71% of the planet's surface and contain large amounts of methane in their sediments, the role of the marine environment is considered to be small (Bange et al. 1994). Shelf regions comprise only 7.5% of the oceans' surface areas, but seem to be of great importance for oceanic methane emissions due to high methane production rates in the sediment and a fast ventilation of the relatively shallow water column (Schmale et al. 2005, 2010a,b). In these systems, the main part of methane is generated under anoxic conditions within the sediment by methanogenic Archaea (Reeburgh 2007).

Apart from this sedimentary source, the process of methane production in well-oxygenated waters has received considerable attention over recent years (e.g., Karl et al. 2008; Keppler et al. 2009; Damm et al. 2010; Grossart et al. 2011; Repeta et al. 2016; Tang et al. 2016). The paradox of methane accumulation in these relatively shallow waters, commonly termed as “oceanic methane paradox,” has been sporadically observed in lakes (Grossart et al. 2011) as well as in marine ecosystems such as the Gulf of Mexico (Brooks et al. 1981), the Black Sea (Schmale et al. 2010a), the Baltic Sea (Schmale et al. 2012), Arctic waters (Damm et al. 2015), or above the continental shelf off the coast of Spain and Africa (Traganza et al. 1979). Even though this phenomenon has been described in the literature over the last decades, the potential sources of shallow methane accumulation are still controversially discussed (Karl et al. 2008; Keppler et al. 2009; Damm et al. 2010; Grossart et al. 2011; Tang et al. 2016). At present, we are far from a detailed process understanding that would
allow determination of the factors, which support and steer the methane production. Recent studies relating to this topic suggest that methane production was either related to the C-P-lyase pathway from methylphosphonate (MPn), in particular under phosphate-stressed conditions (Karl et al. 2008; Repeta et al. 2016), or to the activity of potentially methanogenic Archaea attached to photoautotrophs (Grossart et al. 2011). Alternatively, it is suggested that methane can be produced under anoxic conditions by the degradation of dimethylsulfoniopropionate (DMSP) or its degradation products (Damm et al. 2008, 2015). Subsequent studies propose that DMSP may serve as a carbon source and that the associated biochemical conversion of methylated compounds to methane can provide energy (Damm et al. 2010). A new laboratory approach with the coccolithophore Emiliania huxleyi showed that marine algae are also capable of producing methane under controlled laboratory conditions (Lenhart et al. 2016). An older theory attributes the microbial formation of methane to anoxic microniches within decaying organic aggregates or zooplankton fecal pellets (Oremland 1979; Karl and Tilbrook 1994; Holmes et al. 2000). De Angelis and Lee (1994) formulated the copepod-gut flora theory based on their observations in which methane production was observed during zooplankton grazing in lab-experiments. Their study showed that methane production is copepod species-specific and, to a lesser degree dependent on the phytoplankton species being grazed.

In the Baltic Sea, a semi-enclosed brackish basin connected to the oceanic waters of the North Sea, methane concentrations in the water column usually increase with water depth, indicating a release of methane from the sediment into the water column (Schmale et al. 2010b). The highest concentrations of methane were identified in the deep basins of the central Baltic Sea with concentrations up to 1086 nM (Landsort Deep). However, in the Baltic Sea, the methane flux from the deep methane pool toward the sea surface is strongly hampered by microbial methane oxidation within the oxic/anoxic transition zone below the permanent halocline (Berndmeyer et al. 2013; Jakobs et al. 2013; Schmale et al. 2016). Continuous equilibrator measurements of the concentration of methane in the surface water conducted in the Baltic Sea indicate that the formation of a thermocline during the summer leads to a buildup of a methane pool below this upper density boundary (Gülzow et al. 2013). The wind-induced mixing of the water column during autumn and winter ventilates the accumulated methane upward resulting in elevated, though small, methane fluxes into the atmosphere in the central Baltic Sea (Gülzow et al. 2013). Apart from the sedimentary methane source, vertical water column concentration-profiles of methane and its stable carbon isotope indicate an in situ biogenic methane production below the thermocline ($\delta^{13}$C-CH$_4$ = $-59.9\%_o$; atmospheric equilibrium = $-47.4\%_o$), which may have a more important impact on the atmospheric methane flux due to its shallower source-location (Schmale et al. 2012; Jakobs et al. 2014).

The Baltic Sea is a highly productive ecosystem where methane maxima in the upper well-oxygenated water column have been repeatedly recorded during the summer months (Schmale et al. 2010b, 2012; Jakobs et al. 2013; Gülzow et al. 2014). In the present work, we examine the methane enrichment in the upper water column of the central Baltic Sea (eastern Gotland Basin [EGB] and western Gotland Basin [WGB]) in July 2012 and combine methane gas chemistry with plankton studies and microbiological investigations to discuss the potential parameters that may influence the methane dynamics in the oxygenated waters. In the EGB, which showed a pronounced shallow methane enrichment in contrast to the station in the western part of the basin, additional incubation experiments on a copepod-dominated seston fraction were conducted to identify and quantify zooplankton-associated methane production rates. Finally, we developed mass balances of diffusive methane fluxes to quantify methane sources and sinks below the thermocline and at the sea–air interface to range the estimated contribution of zooplankton-associated methane production to the maintenance of the subthermocline methane accumulation and to the measured flux of methane at the sea–air interface.

### Methods

On expedition M87/4 between 27th June 2012 and 22nd July 2012, on board RV Meteor, we performed methane concentration measurements in the water column to identify areas characterized by methane enrichments of different intensities. Based on these areal investigations, we defined two main stations, the first in the WGB and the second in the EGB (Fig. 1). These were then studied analytically with different approaches to discuss the parameters influencing the production of methane in the shallow oxygenated water column. Additional samples for microbiological studies of methanogenic organisms were taken on expedition 06EZ1213 of RV Elisabeth Mann Borgese at the same stations between 25th July 2012 and 7th August 2012.

### Physical characterization of the water column

For the investigations of the physical properties of the water column during M87/4, we performed studies in the upper 100 m with an SBE 911plus conductivity, temperature, depth (CTD) system (Sea-Bird Electronics, U.S.A.) consisting of a SBE 9plus CTD unit with redundant sensor packs for CTD and oxygen and a SBE 11plus deck unit. Fluorescence was measured with a FLNTURTD sensor from Wet Labs (U.S.A.). Real-time data acquisition and post-measurement data processing were performed using the SBE Seasoft V2 software package. The sensors were mounted on an SBE 32 carousel water sampler with a set of 24 × 10 L free-flow bottles from HydroBios (Germany) for water column sampling. A similar system was used on RV Elisabeth Mann Borgese in August 2012.
In addition, high-resolution microstructure measurements were undertaken with a free-falling MSS90-L microstructure profiler (SN 055) to obtain vertical eddy viscosity profiles. The instrument was equipped with precision CTD sensors for pressure, temperature, and conductivity, two airfoil shear probes (PNS06), and a SeaPoint turbidity sensor. Sampling frequency was 1024 Hz for all sensors. The instrument was operated in free-falling mode with a sinking velocity of 0.5–0.6 m s$^{-1}$. The two shear probes provided estimates of the vertical current shear on a millimeter-scale, from which the dissipation rate of turbulent kinetic energy (TKE) was derived. The vertical eddy diffusivity or eddy viscosity ($A_z$) was estimated according to Osborn (1980) as follows:

$$A_z (m^2 s^{-1}) = \frac{\Gamma \times \varepsilon}{N^2}$$

where $A_z$ is the vertical eddy diffusivity, $\Gamma$ is the mixing efficiency, $\varepsilon$ is the TKE dissipation rate, and $N$ is the stratification of the water column expressed as Brunt-Väisälä frequency. For the mixing efficiency $\Gamma$, the commonly accepted value of 0.2 was used. The technical details of the microstructure probe (MSS) profiler and for the data processing are described in Prandke and Stips (1996) and Prandke et al. (2000).

The vertical eddy viscosity profiles were combined with a high resolution methane profile taken on 18th July to exemplarily calculate the vertical turbulent methane transport in the EGB (see “Discussion” section). The corresponding four microstructure profiles were gathered within 2 h after the water sampling for methane analysis. To minimize the impact of short-term vertical displacement by internal waves, averaging of the eddy viscosity profiles and fitting to the CTD profile was performed along the density vector.

**Water column methane analysis**

Methane concentrations and their stable carbon isotope ratios were analyzed to identify methane enrichments in the upper water column and to discuss their origin. Water samples were collected in high resolution in the upper water column every 3 m with the carousel water sampler. For methane concentration measurements, water samples (600 mL) were taken directly from the free-flow sample bottles and transferred into pre-evacuated 1100 mL glass bottles. Within 3 h after sampling, the dissolved methane was extracted from the water samples, using a partial vacuum extraction line, and subsequently analyzed with a gas chromatograph equipped with a flame ionization detector.
Surface-water methane concentration and sea-air fluxes

During the entire cruise, we employed a system for the continuous measurement of the partial pressures of methane and carbon dioxide based on coupling of an off axis integrated cavity output spectrometer to an air–water equilibria unit. Only the methane measurements are used in this study. The principle of operation, the sensor/equilibrator setup, and further data processing have been described in Gülzow et al. (2011). In this study, data acquisition of the sensor/equlibrator system data together with the ship’s UTC time stamp and GPS coordinates were undertaken at 0.2 Hz. For T-correction, the data were merged with the ship’s thermostalinograph data, and all data were bin averaged over 1 min. For calibration, we used a standard gas of 1.975 ppm methane in natural air, fine calibrated at the Max Planck Institute for Biogeochemistry in Jena (now integrated carbon observation system [ICOS] Central Analytical Lab, Flask and Calibration Lab), which was analyzed several times during the cruise. Between 30th June and the end of the cruise, the Calibration Lab, which was analyzed several times during the sampling time of the microbiological samples was performed using a purge and trap method, see Jakobs et al. (2014).

Plankton studies

Phyto- and zooplankton samples were used to identify differences in the plankton community structure between the two study sites that could help to explain variations in the development of the shallow methane anomaly (de Angelis and Lee 1994). Phytoplankton was sampled in the upper water column in both basins (WGB down to 15 m and EGB down to 45 m water depth) with the carousel water sampler from discrete depths. The water was filled into glass bottles (200 mL), preserved with 1 mL acetic Lugol solution and analyzed for phytoplankton composition and cell-carbon biomass according to the Utermöhl method as described in the manual of HELCOM (2012).

Zooplankton was sampled from both basins above the thermocline (20–0 m) using a WP2 net (mouth opening 0.25 m², mesh size 100 μm) during nighttime according to HELCOM (2012). The zooplankton accumulation below the thermocline and the dial vertical migration of the zooplankton organisms were monitored with hydroacoustic studies using a broadband R2SONIC 2024 with 200–400 kHz frequency and variable pulse length (Cox et al. 2013). These studies were targeted on the seston fraction > 100 μm, typical for the Baltic Sea zooplankton (HELCOM 2012). To cover the entire cruise period, we recorded the back scatter signal by means of continuous video monitoring. From these video data, we extracted 5 min mean profiles of the water column down to 105 m. The upper 6 m were discarded to remove the ship disturbances. Due to variable weather/ocean conditions, we had continuously to recalibrate the back scatter signal to keep the signal-to-noise ratio constant. Therefore, the resulting data allow for qualitative analysis only.

Microbiological investigation

Microbiological studies were conducted on samples collected on expedition 06EZ1213 on RV Elisabeth Mann Borgese in the WGB and EGB to identify methanogenic Archaea in the shallow oxygenated water column.

Water samples of 1 L were filtered onto 0.22 μm pore-size polycarbonate filters (Millipore GVWP, 47 mm diameter, Darmstadt, Germany), immediately frozen in liquid nitrogen and subsequently stored at −80°C until further analysis. For the nucleic acid extraction, samples from the upper water column that showed highest methane concentrations were used. For analyses in the EGB, a filter sample from 50 m depth (8.9 nM of ambient methane) and for the WGB, a filter sample from 40 m and 50 m depth (10 nM of ambient methane) were used.

DNA was extracted from the filters with an acidic phenol extraction (Weinbauer et al. 2002), using 30 μL of diethylpyrocarbonat (DEPC) H₂O₂ in the final elution step. The DNA concentration was measured with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, Delaware, U.S.A.). For the generation of Archaea specific 16S ribosomal ribonucleic acid (rRNA) polymerase chain reaction (PCR) products, 1 μL of DNA (~ 40 ng) was combined with 1 μL of 10 pmol μL⁻¹ Met630F and Met803R primers (Hook et al. 2009), 0.125 μL Taq Polymerase (Thermo Fisher Scientific, St. Leon-Rot, Germany), 2.5 μL 10× PCR buffer (Thermo Fisher Scientific, St. Leon-Rot, Germany), 0.5 μL 40 mM dNTP mix (Thermo Fisher Scientific, St. Leon-Rot, Germany), 2.5 μL 25 mM MgCl₂ (Thermo Fisher Scientific, St. Leon-Rot, Germany), and 15.875 μL sterile nuclease-free...
water with a total reaction volume of 25 μL. The following PCR conditions were used: initial denaturation at 95°C for 15 min, followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 30 s and extension at 72°C for 1 min, finishing with a final extension of 72°C for 10 min.

The PCR products were analyzed on a 1.2% agarose gel, stained with ethidium bromide (Boehringer Mannheim, Mannheim, Germany), to confirm the presence of a single, correctly-sized band. To clean the PCR products the Nucleo-SpinGel and PCR Clean-Up kit (Macherey-Nagel, Düren, Germany) was used following the manufacturer’s instructions. 16S rRNA gene fragments were cloned using StrataClone PCR Cloning Kit (Agilent Technologies, Waldbronn, Germany) as described in the manufacturer’s procedures. From each clone library, random colonies were picked and for a total of 96 colonies the 16S rDNA gene insert was sequenced with the vector specific primer M13-24F-BLUE, using the MTP Sanger sequencing service (LCG, Berlin, Germany). The resulting sequences where checked afterward for chimeras with the DECIPHER’s Find Chimeras web tool (Wright et al. 2012).

All sequences were submitted to the European Nucleotide Archive under accession numbers LT671514 through LT671576 (see Supporting Information Table 1). Raw sequence data was analyzed using BioEdit Sequence Alignment Editor (Hall 1999) and vector fragments were removed from each end of the sequence. All good quality sequences were aligned using the SINA Online Aligner (SILVA Incremental Aligner, v1.2.11) in the web interface of www.arb-silva.de/aligner/ according to Pruesse et al. (2012). The aligned partial clone sequences (~190 bp) were added to the existing All-Species Living Tree (LTPs123, September 2015, Yarza et al. 2008) using parsimony in arb (version 6.0.2.; Ludwig et al. 2004).

Incubation experiments

Incubation experiments were carried out at the EGB station, to test our hypothesis that methane enrichments in the shallow oxygenated waters (in about 30 m water depth) are connected to a copepod-dominated seston fraction (>100 μm). Animals for the experiments were sampled in the layer between 25 m and 45 m, where a maximum concentration of methane and seston particles was observed during daytime by the hydroacoustic measurements (see “Results” section). Specifically, we used a WP2 net (mouth opening 0.25 m², mesh size 100 μm; HELCOM 2012) equipped with a nonfiltering cod end that was gently towed (0.1 m s⁻¹) from 45 m to 25 m, closed and brought onboard. We pooled seston samples from a total of four tows for the incubation experiments to avoid dropping below the detection limit for the methane concentration measurements with our gas chromatographic method (0.1 nmol). After each tow, the animals were gently poured, without rinsing, into a 10-liter bucket filled with sea water from 35 m depth that was obtained from the prior CTD cast. After 1 h, a subsample of 3450 mL was decanted from the bucket into a 4000 mL beaker to reduce the amount of dead animals and particles. Fifty milliliters of this highly concentrated seston suspension was conserved in 4% buffered formaldehyde to analyze the zooplankton community structure in the 45–25 m layer, and the rest of the sample was used for the methane production experiments (see process chart in Fig. 2).

For the incubations, we prepared four different seston dilutions with increasing adult T. longicornis densities from the seston suspension sampled and concentrated from the 45–25 m depth layer. We quickly and gently added 2 × 3780 adult T. longicornis (equivalent to 2 × 300 mL of seston suspension, Bottle 5 and 6), 3 × 6300 adult T. longicornis (equivalent to 3 × 500 mL of seston suspension, Bottle 7, 8, and 9), 1 × 7560 adult T. longicornis (equivalent to 1 × 600 mL of seston suspension, Bottle 10), and 1 × 8820 adult T. longicornis (equivalent to 1 × 700 mL of seston suspension, Bottle 11) to 2.3 L polycarbonate bottles (Nalgene®) using a 1 L graduated cylinder. The bottles had been half-filled with water from 35 m depth before the animals were added, were filled completely after the respective animal suspension was added and were subsequently closed with Teflon coated butyl-rubber septa caps (Montoya et al. 2004; Voss et al. 2006). The vitality of the animals was controlled daily by visual inspection of the animals’ movement in the incubation bottles and the absence of detritus accumulations on the bottom of the bottles. A sterile filtered control sample (filter size 0.2 μm, Bottle 1 and 2) as well as a natural water sample obtained from 35 m water depth (including undefined microplankton and particles of a size <100 μm, Bottle 3 and 4) were included in this incubation experiment. A defined headspace volume of 50 mL (atmospheric air) was added to the gas-tight sample bottles and the bottles were stored in the cold store at in situ temperature (about 6°C). The bottles were gently shaken several times over the day to limit the development of an oxygen gradient in the bottle. Methane concentrations were measured daily by taking a gas sample (1 mL) from the headspace and determining the methane mole fraction in the headspace by gas chromatography (for method details, see above and Schmaler et al. 2010a). A pressure balance was achieved by the injection of an equivalent injection of filtered sea water. The total methane content in the incubation bottles was calculated using the solubility coefficients established by Wiesenburg and Guinasso (1979). At the beginning of our experiment, the water samples were equilibrated with the head space of the incubation bottles and the methane concentration was then determined. This value was then subtracted from the concentrations measurements that followed in order to obtain the methane accumulation over the course of the experiment for each incubation bottle. Oxygen concentrations in the incubations were controlled during the experiment using an oxygen microprobe (Microx TX3, PreSens, Germany) to verify an oxic environment.
Mass balance approach

We developed a mass balance to assess if methane production rates from *T. longicornis* can maintain the methane enrichment below the thermocline and the flux at the air-sea interface. These calculations were exemplarily conducted on the dataset obtained on the 18th July, comprising of a high resolution methane profile, a high resolution eddy diffusivity profile, and methane production rates derived from our seston incubation experiments. The vertical methane transport ($T_{CH4}$) was estimated based on a nonadvective Fickian turbulent diffusion model on the gradient of measured methane concentration ($\frac{\partial C_{CH4}}{\partial z}$) and vertical eddy viscosity ($A_v$).

$$T_{CH4}(m^2 s^{-1}) = A_v \times \left( \frac{\partial C_{CH4}}{\partial z} \right)$$

In this model, the upward (downward) directed transports are defined as positive (negative) values. Since our approach focuses on processes that sustain the prominent subthermocline methane peak, calculations were based on a smoothed methane profile that neglects fine scale methane fluctuations. Assuming quasi steady conditions and no lateral fluxes, the divergence/convergence of the methane transport profile has to be balanced by local sources/sinks of methane. The net sources and sinks of methane ($P_{CH4}$) in the water column were calculated as divergence of the estimated vertical methane transport as follows:

**Fig. 2.** Process chart for the set-up of the incubation experiments.
Results

Physical properties of the water column

The hydrographic conditions at the two main stations were characterized by the typical summer season stratification of the Baltic. The upper mixed layer had a temperature of about 16°C, and was separated from the underlying cold intermediate water (about 4°C) by a strong seasonal thermocline between 15 m (WGB) and 25 m (EGB) depth. The halocline at 50–70 m depth represents the transition to the oxygen depleted deep-water layer of the central Baltic. Despite this general structure the particular stations in the eastern and WGB depicted some important differences (Fig. 3). In the EGB, a double thermocline was found, with steep gradients at 15 m and 25 m. This was caused by strong drop in wind speed 2 d before the sampling. The reduced mixing depth together with high surface heat flux established the secondary thermocline at 15 m depth. In contrast, in the WGB, the temperature gradient was weaker. Thus, the transition between the mixed layer and the winter water was smoother than in the EGB.

The strong stratification in the EGB was also reflected in the eddy viscosity profile (Fig. 4). Due to the low wind speed, the eddy viscosity was weak in the surface layer \(10^{-4} \text{ m}^2 \text{s}^{-1}\). Below the surface mixed layer the dissipation of TKE was very low and close to the noise level of the MSS. Thus, the estimated eddy viscosity (Fig. 4) is an upper limit of the true value. The strong stratification at the thermocline reduced the eddy viscosity below 15 m by two orders of magnitude to \(10^{-6} \text{ m}^2 \text{s}^{-1}\). At the halocline, a further drop in eddy viscosity to \(10^{-7} \text{ m}^2 \text{s}^{-1}\) was observed.

Water column methane distribution

At both stations, the surface-water methane concentrations and the stable carbon isotope ratios of CH\(_4\) (\(\delta^{13}\text{C-CH}_4\)) were close to atmospheric equilibrium (\(\sim 3 \text{ nM}, -47\text{%o}\); Fig. 3). However, strong differences between these two stations were observed in the methane and \(\delta^{13}\text{C-CH}_4\) depth profiles. In the WGB, the vertical methane concentration profile was quite stable with maximum concentrations of 7.7 nM at 63 m water depth. The methane profile obtained on 18th July in the EGB showed a strong increase from the sea surface to 17.1 nM below the thermocline at 27 m water depth. This distinct concentration anomaly came along with a shift in the stable isotope values toward light \(\delta^{13}\text{C-CH}_4\) values of \(-67.6\text{%o}\). In contrast, \(\delta^{13}\text{C-CH}_4\) values in the WGB were rather uniform with maximum \(-12\text{%o}\) enrichment at 44 m water depth (\(\delta^{13}\text{C-CH}_4\) of \(-52.2\text{%o}\)).
depth and constantly persisted during the field days (Fig. 5, detected on 10 working days between 5th July and 18th July), although strong variations in the methane concentrations occurred (range of 15–77 nM).

**Surface-water methane concentration and sea–air fluxes**

We compiled all data gathered within one nautical mile of the station in the EGB during the entire cruise, summing up to a total of 8189 data points (minute means) recorded between July 7th and 20th. Surface-water methane concentrations were very constant over the entire campaign, ranging between 3.1 nM and 3.7 nM, with an average value of 3.45 nM. Data was bin averaged for 1 h intervals to scrutinize the data set for any diurnal trend. No time interval was represented by less than 200 values. The mean hourly values showed a general slightly decreasing trend between 3 a.m. and 5 p.m., a stagnant phase between 5 p.m. and 8 p.m., and an increase from 9 p.m. to 3 a.m., though some deviations from this trend occurred on an hour to hour basis (Fig. 6a). The difference between the maximum mean concentration at 3 a.m. and the minimum at 5 p.m. was ~ 0.105 nM or 105 pM.

The mean equilibrium concentration derived from the same data set was 2.93 nM. The mean oversaturation of 18% is in line with data from the summer situation in the

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*Fig. 4.* Dataset obtained in the EGB on 18th July for the set-up of the mass balance: (a) eddy viscosity (green line) and vertical methane concentration profile (black dots, individual measurements; black line, smoothed profile). (b) Methane transport ($T_{CH_4}$, blue line) and methane production ($P_{CH_4}$, red line).

*Fig. 5.* Compilation of methane profiles obtained in the EGB between 5th July and 18th July.
Gotland Basin in 2010 reported in Gülzow et al. (2013), displaying an oversaturation of ~ 20%. The averaged sea–air flux was 1.90 μmol m⁻² d⁻¹. The variation in the mean hourly fluxes is larger than that of the surface concentration, reflecting the stronger variation of wind speeds (Fig. 6b).

**Plankton community**

Our hydroacoustic investigations in the EGB revealed dynamic changes in the position of the layer with highest seston > 100 μm accumulation (Fig. 7). During daytime, we identified the maximum density layer of seston > 100 μm below the thermocline, at a water depth of 25–45 m. This coincided with the location of the methane concentration peak (Figs. 3, 5) and a mesoplankton community > 100 μm that was dominated by different stages of *T. longicornis* (Fig. 8c). The copepod *T. longicornis* has been found to perform dial vertical migration between 40 m (daytime) and 10 m (nighttime) in the central Baltic Sea in August (Hansen et al. 420
2006). Assuming that a dial vertical migration of *T. longicornis* was largely responsible for the change in the position of the seston > 100 μm layer (Fig. 7), the hydroacoustic data indicates that the copepods started to enter into the mixed layer depth at about 9 p.m. and returned below the thermocline at about 2 a.m. (Fig. 7).

A comparison of the nighttime mixed layer zooplankton communities from the EGB and the WGB revealed the dominance by *T. longicornis* at the EGB station, while *Acartia* spp. dominated at the WGB station (Figs. 8a,b). At the EGB station, the nighttime surface zooplankton community from 9th July at the EGB station (Fig. 8b) was almost congruent to the community found in the 45–25 m layer (sampled 18th of July, Fig. 8c) and subsequently used for the incubation experiments (Fig. 8c).

The highest chlorophyll a concentration was within the upper 20 m of the water column (Fig. 3). This was confirmed by the biomass data of the phytoplankton as shown in Fig. 9. Even though in the WGB only the upper 15 m were sampled, we assume that the phytoplankton concentration followed the shape of the fluorescence profile and decreased below 12 m water depth as observed in the EGB (Figs. 3, 9). It became evident that the dinoflagellate genus *Dinophysis* was almost exclusively represented by *Dinophysis norvegica*, and that the abundance of this plankton species was higher in the EGB compared to the WGB. *D. norvegica* was concentrated at a depth of 10–20 m, and represented up to 79% of the dinoflagellates in the WGB (at 15 m water depth), and 97% in the EGB (at 20 m water depth). Other groups such as Prymnesiales and Cyanobacteria (Nostocophyceae) seemed to prefer the upper mixed layer.

**Identity of methanogenic Archaea**

16S rRNA gene analyses based on DNA filters from the WGB and EGB revealed that 63 clonal sequences (no chimeras) matched within the domain Archaea. Sixty-two of these...
sequences were spread throughout the Archaea, outside of the known methanogenic clusters (Fig. 10): 15 of these sequences clustered with the clades *Thermoplasmatales* and *Methanomassiliicoccaceae*, with 11 retrieved from the WGB and four from the EGB, respectively. The other 47 clonal sequences clustered with the clade *Nitrososphaeraceae*, with

**Fig. 10.** Overview of all archaeal 16S rRNA gene sequences retrieved from the cruise 06EZ1213. All sequences were added to the All-Species Living Tree (LTP123, Yarza et al. 2008) using the parsimony-tool of arb6. Numbers on leaves show the amount of total sequences within the respective clade and leaves comprised of clonal sequences are highlighted in red. Taxonomic affiliation is represented by different colors: black for Domain, purple for Kingdom, green for Class, orange for Order, and blue for Family.

**Fig. 11.** Methane accumulation in incubation experiments with different seston concentrations. Details of the different treatments are included in the process chart in Fig. 2.
45 sequences retrieved from the WGB and two from the EGB. One 16S rRNA sequence (LT671516), generated from the EGB, was affiliated with the clade Methanomicrobiaceae and thus truly identified as a methanogen.

**Methane production in incubation experiments**

Methane production was observed in all flasks during the incubation experiments (Fig. 11). In all set-ups, the trend of methane accumulation was very similar and reflected highest production rates on the first day and decreasing methane production within the following 2 d. Visual inspection of the animals revealed vivid movement in most flasks, except for bottles 10 and 11 on days 2 and 3 when dead animals started to accumulate at the bottom of the flasks and oxygen levels decreased into a suboxic state on day 2 (concentration of O2 about 50 µM) and anoxic state on day 3 (concentration of O2 below 1 µM). The experiments clearly showed that the seston concentration correlated positively with the methane production rates. Maximum production rates after 1 d were observed in the incubation that contained the highest seston concentration (Bottle 11 with 2.2 nmol CH4 d⁻¹). Also, in the set-up with a natural water sample that included undefined microplankton and particles, methane concentrations increased. Also here, the highest rates were observed during the first day (0.9 nmol CH4 d⁻¹). Similar to the other incubations, methane levels remained nearly constant after the second day. In contrast to these incubations, no methane production was observed in the control flask that contained sterile filtered sea water (filter size 0.2 µm). Because potential artifacts like decreasing oxygen values, food limitation and effects of the unnatural high zooplankton abundance got more pronounced during the course of the experiments, we rely on the observed methane production rates of the first day for further interpretation.

Oxygen concentrations on day 2 of the experiment were on average 295 µM in the sterile filtered (Bottle 1 and 2) and in the natural water sample (Bottle 3 and 4), and 304 µM at the end of the incubation experiment (day 3). Oxygen concentrations in Bottle 5 and 6 declined from 204 µM on day 2 to 173 µM on day 3. A similar trend was observed in Bottle 7, 8, and 9, with a decrease from 136 µM to 77 µM. The strongest oxygen depletion was observed in Bottle 10 and 11 from 50 µM on day 2 to values below 1 µM at the end of the incubation experiment.

**Discussion**

**Potential sources of methane in subthermocline oxic waters**

Seasonal methane studies in the central Baltic Sea conducted over a time frame of 2 yr showed that methane concentrations in the upper water column were always highest during August (Jakobs et al. 2014). The strongest methane enrichment in the shallow oxygenated water column was observed in the eastern Gotland Sea with a concentration up to 27 nM at a 40 m water depth in the year 2011 and 25 nM at 50 m in 2012. In contrast, methane concentrations in the western Gotland Sea were relatively low with concentrations of about 10 nM at a 50 m water depth in both years. These observations are in accordance with this study, which was conducted in July 2012 (Figs. 3, 5). The high sampling resolution applied in our study showed that the methane enrichment was only developed in a narrow band. Thus, the concentration peak can be easily missed. Lower concentrations above and below this layer hint at a source at this depth level. The stable carbon isotope ratios of methane in the EGB clearly indicate it’s in situ biogenic production (−67.6‰; Fig. 3, Reeburgh 2007). Jakobs et al. (2014) showed that methane oxidation within the oxic/anoxic transition zone at about 100 m water depth leads to a ¹³C enrichment resulting in δ¹³C values of about −40‰ in this specific zone in both basins. Since our studies show δ¹³C values of about −67.6‰ within the methane concentration peak, we can exclude that methane from the ¹³C-enriched deep pool contributes to the shallow methane enrichment.

Different methane sources in the oxygenated water column are currently under debate (de Angelis and Lee 1994; Karl et al. 2008; Keppeler et al. 2009; Damm et al. 2010; Grossart et al. 2011). We assume that subthermocline methane production by the microbial break-down of MPn, as proposed for phosphate-stressed environments (Karl et al. 2008), played a rather subordinate role in the free water column, as phosphate (PO₄) was available below the thermocline during our time of sampling (PO₄ conc. ∼ 0.5 µM; Nausch et al. 2013, 2016). Laboratory experiments showed that the uptake of phosphate is preferred during the microbial growth when phosphate and MPn are concurrently available (Karl et al. 2008; Beversdorf et al. 2010), and methane production was inhibited when inorganic phosphate was added to seawater amended with MPn containing semi-labile dissolved organic matter (DOM) (Repeta et al. 2016). Furthermore, the δ¹³C-CH₄ value of −67.6‰ detected in the methane concentration maximum (Fig. 3) in our study goes against a relevant contribution of methane through MPn-decomposition. For methane released from MPn, Repeta et al. (2016) report δ¹³C-CH₄ values of −39‰. These values are considerably more enriched in ¹³C than methane that is typically associated with anaerobic methanogenesis (−50‰ to −110‰; Whiticar 1999). The strong discrimination against ¹³C displayed in our vertical methane profile suggests the reduction of CO₂ by Archaea (Whiticar 1999; Valentine et al. 2004). Other studies indicate that methane production in the oxygenated surface water is associated with primary production (Bogard et al. 2014; Tang et al. 2014), but the low concentration of phytoplankton biomass below 20 m water depth (Fig. 9) rules out a major contribution of algal linked methanogenesis to the subthermocline methane enrichment. For instance, Grossart et al. (2011) suggest that the attachment of photoautotrophs to hydrogenotrophic methanogens
allows anaerobic growth and a transfer of diazotroph-derived hydrogen between these microorganisms. Our dataset displays a spatial separation of the Chl a maxima (Fig. 3) and phytoplankton biomass maxima (Fig. 9) above the thermocline from the methane maximum below the thermocline (Fig. 3) that clearly points to a methanogenic source that, in contrast to the Grossart et al. (2011) study, was not congruent with phytoplankton density. Bogard et al. (2014) found indications for an algal-driven acetoclastic methane production in surface waters, a process that appears to be of minor relevance during our studies since the light δ13C-CH4 values detected within the methane peak can hardly be explained by acetoclastic methanogenesis that only shows relatively small isotope fractionation effects (between −5‰ and −35‰, Goevert and Conrad 2009). Another potential source for methane in the water column is the reduction of DMSP (Damm et al. 2010), an algal osmolyte that is abundant in marine phytoplankton and enriched in the mixed layer (Matrai and Keller 1993). Anaerobic methane production by the microbial metabolism of DMSP and its degradation products (e.g., methanethiol) is known from anoxic sediments (Kiene et al. 1986; Finster et al. 1992; van der Maarel et al. 1995). The associated methyl reductase Mcr gene complex was so far only detected in methanogenic Archaea. In the present analysis, we could not detect any known Archaea that metabolize MPn, though further analysis is needed. Nevertheless, methane production could be detected in microms experiments with DMSP spiked oxygenated water, in which the abundance of Archaea was negligible (Damm et al. 2010). To explain these contradictory results, Damm et al. (2015) developed a theoretical approach to demonstrate that bacteria are able to maintain an anoxic cytoplasm in which the DMSP metabolism might take place. Recent works showed that microalgae might also contribute to surface-water methane supersaturation by demethylation of organosulfur compounds (Lenhart et al. 2015). However, methane formation from methionine mediated by coccolithophores as described from laboratory experiments (Lenhart et al. 2016) can be excluded, since calcifiers are absent in the central Baltic Sea (Tyrrell et al. 2008).

According to this line of reasoning, we assume that the main contribution for subthermoline methane came from its production in anoxic microniches within particulate biogenic material (e.g., fecal pellets; Karl and Tilbrook 1994) or gastrointestinal tracts of zooplankton (de Angelis and Lee 1994). This assumption is supported by 16S rRNA sequences generated from the EGB, clustering with the clade Methanomicrobiaceae (Fig. 10). The family of Methanomicrobiaceae uses H2/CO2 or formate as a major energy source and some species also use primary or secondary alcohols as an electron donor for the methanogenesis (Oren 2014). Several members of the Methanomicrobiaceae have been described in marine sediments such as Methanogenium marinum, Methanolacinia paynteri, Methanogenium marisnigri, or Methanogenium cariaci (Romesser et al. 1979; Oren 2014). For the WGB, we could not detect any methanogens based on our 16S rRNA approach. However, the present analysis was conducted on a relatively small scale and therefore, it is possible that other methanogenic Archaea were undetected due to the methodological approach rather than their actual absence. Based on our hydroacoustic findings, which showed that during the daytime the density of the mesozooplankton fraction was highest in the depth interval where methane was enriched, we propose that microniches in the guts of copepods or their faeces may host the methanogenic Archaea. This hypothesis obtains support from the fact that putative hydrogenotrophic (Methanogenium, Methanobacterium) and methylotrophic (Methanolobus) methanogens have already been identified in fecal pellets of copepods (Cynar and Yayanos 1991; Marty 1993; Ditchfield et al. 2012). In addition, Bianchi et al. (1992) observed methane production associated with both copepods and egested fecal pellets, which would be consistent with the presence of methanogens in the gut of copepods. The authors speculated that methanogens, active within fecal pellets originated from the digestive tract of the zooplankton and are incorporated into the pellet at the time it is produced.

The anoxic potential of different marine aggregates was investigated in detail by Ploug et al. (1997, 2008), Ploug and Jørgensen (1999), and Ploug (2001). According to their oxygen microsensor studies, only “carbon and nutrient rich” aggregates > 600 μm in size fulfill the prerequisite to become an anoxic microenvironment. In a study by Ploug et al. (2008), the oxygen environment in small copepod fecal pellets (88–168 μm long, 40 μm wide) was suggested to be too unstable for the relatively slow growth of obligatory anaerobic microorganisms such as methanogenic Archaea. Furthermore, Ploug et al. (1997) used Archaea specific primers but could not identify methanogens in copepod fecal pellets from experimental Acartia tonsa grazing on the cryptophyte Rhodomonas baltica. Their results from the Acartia study are in contrast to the study by Ditchfield et al. (2012) that identified 16S rRNA sequences in T. longicornis and Acartia clausa fecal pellets closely related to methanogens.

De Angelis and Lee (1994) conducted laboratory experiments to study the potential of different copepod species to produce methane under oxygenated conditions. This study identified T. longicornis as a key player in zooplankton associated methane production. Their finding strongly supports our hypothesis, since T. longicornis was the dominant copepod species in the EGB (Fig. 8), the area where methane was enriched in the upper oxygenated water column (Fig. 3). In contrast to the EGB, the abundance of T. longicornis in the WGB was relatively low and the copepod community was dominated by Acartia spp., an animal that did not produce any methane in the experiments carried out by de Angelis and Lee (1994). De Angelis and Lee (1994) further studied the influence of the food source on methane production
with the outcome that *T. longicornis* had their highest production rates when the diet consisted of dinoflagellates (*Prorocentrum minimum*). The influence of the diet on the redox conditions in copepod guts was also supported by oxygen microprofiles (Tang et al. 2011). Interestingly, during the time of our studies, the abundance of the mixotrophic dinoflagellate *D. norvegica* was relatively high within the thermocline in the EGB, whereas the abundance of this phytoplankton species was distinctly lower in the WGB (Fig. 9). Carpenter et al. (1995) showed that in summer *D. norvegica* is the predominant dinoflagellate within the thermocline of the EGB and no cells of this species could be detected in the mixed layer depth. Based on these reports, it appears that the observed methane maxima in the EGB and the absence of such subthermocline methane enrichment in the WGB are likely to be related to the relatively high abundances of *T. longicornis* and *D. norvegica*.

**Subthermocline methane production rates**

Our measurements show that the methane enrichment coincided with the density maxima of mesozooplankton (Figs. 3, 5, 7). To assess methane production rates from this plankton fraction, we applied field-incubations with different seston concentrates obtained from the relevant depth interval. According to our assumption that *T. longicornis* was mainly responsible for the methane increase within the first day in our incubations (Fig. 11), we calculated an average methane production rate of 0.3 pmol CH$_4$ d$^{-1}$ per adult *T. longicornis*. This production is relatively low compared with the rate measurements conducted by de Angelis and Lee (1994), who obtained rates between 3.9 pmol CH$_4$ *T. longicornis* $^{-1}$ d$^{-1}$ and 20.4 pmol CH$_4$ *T. longicornis* $^{-1}$ d$^{-1}$. A factor that might explain the difference in the production rates is the size deviation between *T. longicornis* in the Atlantic Ocean (1300 $\mu$m) that was used in the de Angelis and Lee (1994) study and *T. longicornis* in the Baltic Sea (700 $\mu$m). The copepod size and the extension of the digestive tract influence the oxygen gradient within the gut (Tang et al. 2011), and one could assume that the activity of oxygen sensitive methanogenic Archaea might be suppressed in smaller copepods. However, Tang et al. (2011) could demonstrate that not only the size but also the diet can cause oxygen depleted conditions in the intestinal tract that would allow methane production even in smaller copepods. Other studies indicate that the integrity of the peritrophic membrane, that encloses the food within the copepod gut and surrounds the fecal pellets, also influences oxygen diffusion and that an intact membrane allows oxygen depleted conditions already within 100 $\mu$m (Allardreg and Cohen, 1987). Investigations on methanogens showed that these microorganisms can be metabolically active under oxygenated conditions by overexpressing oxygen detoxifying genes, which was demonstrated for biological soil crust samples (Angel et al. 2011). We think that food limitation was the most probable cause for the low methane production rates in our experiment. This assumption was supported by the reduced phytoplankton biomass concentrations below 20 m depth and by the very low biomass below 30 m depth compared to the phytoplankton biomass in the mixed layer (Fig. 9b). While we probably underestimated the rate of methane production in our incubation experiment, a positive association between methane production and the abundance of *T. longicornis* was evident (Fig. 11).

We also observed relevant methane production rates in the incubation of the natural water sample obtained from 35 m water depth, for which sources, other than copepods, must be responsible, since mesozooplankton was not observed in these approaches. It might be speculated that during these incubations, methane was also produced by methanogenesis within anoxic microniches in particles (such as fecal pellets, Karl and Tilbrook 1994). For instance, studies on fecal pellets showed that these aggregates can be highly enriched in DMSP (Kwint et al. 1996) and that the descent of pellets can play a critical role in the vertical flux of DMSP (Daly and DiTullio 1996). Tang (2001) showed that within 24 h up to 95% of the DMSP in freshly produced fecal pellets, is lost by microbial turnover and diffusion from the pellet into the surrounding water. Thus, within anoxic microniches of sinking fecal pellets, methane production from DMSP by Archaea may also have taken place over the course of our experiments and might contribute to the methane enrichment below the thermocline.

**A mass balance to describe sources and sinks of methane in oxic waters**

Our mass balance calculations estimated a maximum vertical methane transport of about $-4 \times 10^{-6}$ $\mu$mol m$^{-2}$ s$^{-1}$ closely below the methane peak at 28 m depth (Fig. 4), which was driven by the strong methane concentration gradient. The upward transport at the upper edge of the peak was considerably lower (1 $\times 10^{-6}$ $\mu$mol m$^{-2}$ s$^{-1}$), since the strong thermocline reduced the eddy diffusivity. Further, the results show the expected surplus of production for the methane peak at 28 m. The integrated methane net production rate (sources minus sinks) in the depth interval between 26 m and 29 m, where the methane enrichment was detected, is 0.33 pmol d$^{-1}$ m$^{-2}$ (i.e., 110 pmol L$^{-1}$ d$^{-1}$). Since the concurrent methane consumption (oxidation) rates are unknown, a calculation of absolute production is not possible and the transport-derived production rate displays a somewhat lower limit.

During our field studies, the abundance of *T. longicornis* was 3.5 individuals L$^{-1}$ on average in the depth interval between 20 m and 60 m. Based on the rate of 0.3 pmol CH$_4$ d$^{-1}$ per adult, *T. longicornis* determined in our experiments, this results in a zooplankton-associated methane production of about 1 pmol L$^{-1}$ d$^{-1}$. Therefore, methane production rates from our incubation experiments are two orders of
magnitude lower than predicted from our mass balance approach (110 pmol L\(^{-1}\) d\(^{-1}\)), and it must be assumed that also other methane sources are necessary to explain the subthermocline methane enrichment or that there is a bias in our budget calculation. As discussed above, a significant discrepancy exists between the rates measured in our experiments and the studies conducted by the de Angelis and Lee (1994) that might be explained by the chosen design of our incubation. Using the production rates determined by de Angelis and Lee (1994, 3.9 pmol CH\(_4\) copepod\(^{-1}\) d\(^{-1}\) and 20.4 pmol CH\(_4\) copepod\(^{-1}\) d\(^{-1}\)), who carried out their experiments under more natural conditions (e.g., natural abundance of copepods), we calculate a zooplankton-associated methane contribution of 14–70 pmol L\(^{-1}\) d\(^{-1}\), indicating that zooplankton has the potential to contribute significantly to the subthermocline methane enrichment. Furthermore, our multibeam survey displays temporal variations in the position and density of the seston >100 \(\mu\)m layer below the thermocline (Fig. 7) that implies a patchy structure of the distribution of zooplankton, a behavior that is well-known from *T. longicornis* (Seuront and Lagadeuc 2001; Dutz et al. 2010). From these observations, it can be expected that the in situ abundance of *T. longicornis* can differ substantially from its average value determined for the 20–60 m depth interval. Such an inhomogeneous distribution will affect the source strength of zooplankton-associated methane production and might explain the variations in the subthermocline methane concentrations observed during the time of our field studies (methane conc. maximum 77 nM and minimum 15 nM).

Apart from these insights into the distribution and strength of the sources of methane, our mass balance approach also delivers critical information about methane sinks in the oxic water column. Our calculations indicate that total production and consumption of methane are balanced in the water column between 20 m and 60 m depth (Fig. 4). Therefore, it can be assumed that methane produced below the thermocline is consumed before it is transported into the mixed layer. Microbial methane consumption within the upper water column has not yet been investigated, but methane oxidation and methanotrophic bacteria were identified in August 2012 within the depth interval of 80–120 m water depth at the oxic/anoxic transition zone of the EGB (Jakobs et al. 2014). Our mass balance suggests that methane oxidation at this depth might not only be maintained through the upward flux of methane from the deep anoxic waterbody but also from the downward flux of methane from the subthermocline methane accumulation. Such a subthermocline balance between sources and sinks of methane most likely exists during the summer months when the upper water column is strongly stratified by a pronounced temperature gradient, a situation that changes in autumn when a decreasing surface-water temperature and an increasing wind stress lead to a destabilization and an upward mixing of water from intermediate depths. Such a ventilation of subthermocline methane is indicated in surface water equilibrator measurements that showed elevated methane saturation values during the August–September period (Gülzow et al. 2013).

Since the transport of methane from below the thermocline into the mixed layer is unlikely to happen during the period of our investigations, the observed methane supersaturation in the surface water (Figs. 3, 6) can only be explained by methane sources that are located in the upper 20 m. Our air–sea flux measurements indicate that an additional source in the mixed layer of 1.9 \(\mu\)mol CH\(_4\) m\(^{-2}\) d\(^{-1}\) (synonymous with an average net production rate of 95 pmol CH\(_4\) L\(^{-1}\) d\(^{-1}\) in the 0–20 m depth interval) is required to maintain the methane loss into the atmosphere. From the vertical migration behavior of *T. longicornis* (Dutz et al. 2010), it can be expected that these copepods also contribute to the methane production while they are grazing in the mixed layer at night (Fig. 7). Such zooplankton related methane production in the mixed layer is supported by the weak diurnal cycle of the methane surface concentrations which shows an increase in methane for the time-interval between 9 p.m. and 2 a.m., during which the zooplankton was present in the mixed layer (Figs. 6, 7). Since the daily residence time of *T. longicornis* in the mixed layer depth is only about 5 h, the methane production rate in the mixed layer of 95 pmol L\(^{-1}\) d\(^{-1}\) appears to be too high to be sustained by zooplankton associated methane production alone.

In contrast to the subthermocline waterbody where the phytoplankton biomass is relatively low (Fig. 9), it is likely that methane sources associated with primary production play a central role in the phytoplankton-rich surface water (Bogard et al. 2014; Tang et al. 2014; Lenhart et al. 2015). Mid-summer blooms of nitrogen fixing cyanobacteria are a common feature in the central Baltic Sea during the time from July to August (Schneider et al. 2014). Different to the subthermocline water, where phosphate is accumulated, nitrogen-fixation can promote upper-ocean phosphate limitation in the central Baltic Sea (Nausch et al. 2008) and it might be speculated that such an environment supports the microbial utilization of MPn resulting in an accompanied release of methane into the surface water (Karl et al. 2008; Repeta et al. 2016).

**Conclusion**

Mechanisms that control zooplankton associated methane production are hitherto largely unknown while the existing knowledge is primarily based upon a limited number of laboratory experiments. Based on our study, it appears that the spatial heterogeneity of methane accumulations in the upper oxygenated water column in different parts of the Baltic Sea might be influenced by changes in the dominant copepod
species and food web structure. However, in contrast to published zooplankton methane production rates, which would have the potential to maintain the subthermocline methane enrichment in the central Baltic Sea, our own incubation experiments suggest that methane production rates obtained from a *T. longicornis* dominated seston fraction are too low to explain the methane enrichment solely by a zooplankton source. For a better process understanding, we propose further field studies to (1) investigate methane production rates of different zooplankton species under more realistic conditions (e.g., natural copepod abundance in incubations), (2) study the differences in the zooplankton-species-specific gut flora, and (3) elucidate the influence of the food source on zooplankton associated methane production. Further investigations are also needed to identify the methane sources that are not related to zooplankton activity and that appear to have an important influence on methane emissions into the atmosphere in the central Baltic Sea. Research so far has mainly focused on a deep process understanding of the individual methane sources; however, it is necessary to develop an integrated scientific approach that assesses the share of each source to surface-water supersaturation in the field.

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Conflict of Interest

None declared.