https://doi.org/10.5194/bg-2019-245
Preprint. Discussion started: 1 July 2019

Biogeosciences Discussions

Methane production by three widespread marine phytoplankton species: release rates, precursor compounds, and relevance for the environment

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Abstract. The world's oceans are considered to be a minor source of methane (CH_4) to the atmosphere although the magnitude of total net emissions is highly uncertain. In recent years the origin of the frequently observed in situ CH_4 production in the ocean mixed layer has received much attention. Marine algae might contribute to the observed CH_4 oversaturation in oxic waters, but so far direct evidence for CH_4 production by marine algae has only been provided for the coccolithophore *Emiliania huxleyi*.

In the present study we investigated, next to *Emiliania huxleyi*, other widespread haptophytes, i.e. *Phaeocystis globosa* and *Chrysochromulina sp*. for CH_4 formation. Our results of CH_4 production and stable carbon isotope measurements provide unambiguous evidence that all three investigated marine algae produce CH_4 per se under oxic conditions and at rates ranging from 1.6 ± 0.5 to 2.7 ± 0.7 µg CH_4 per g POC (particulate organic carbon) d^{-1} at a temperature of 20°C with *Chrysochromulina sp.* and *Emiliania huxleyi* showing the lowest and highest rates, respectively. In cultures that were treated with ^{13}C-labelled hydrogen carbonate $\delta^{13}CH_4$ values increased with incubation time, clearly resulting from the conversion of ^{13}C-hydrogen carbonate to $^{13}CH_4$. The addition of ^{13}C labelled dimethyl sulfide, dimethyl sulfoxide and methionine sulfoxide – known algal metabolites that are ubiquitous in marine surface layers - enabled us to clearly monitor the occurrence of ^{13}C-enriched CH_4 in cultures of *Emiliania huxleyi* clearly indicating that methylated sulphur compounds are also precursors of CH_4. We propose that CH_4 production could be a common process among marine haptophytes likely contributing to CH_4 oversaturation in oxic waters.

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1. Introduction

Methane (CH_4), the second most important anthropogenic greenhouse gas after CO_2, is the most abundant reduced organic compound in the atmosphere and plays a central role in atmospheric chemistry (Denman et al., 2007;Kirschke et al., 2013;Lelieveld et al., 1998). The mixing ratio of CH_4 in the atmosphere has been increasing dramatically from pre-industrial values of about 715 parts per billion by volume (ppbv) to about 1868 ppbv (October 2018, NOAA). The global atmospheric CH_4 budget is determined by the total emission (540-568 Tg CH_4 yr^{-1}) of various sources from terrestrial and aquatic surface areas, that are balanced primarily by one major sink (hydroxyl radicals) in the atmosphere. However, partitioning source categories to reduce uncertainties in the global CH_4 budget is a major challenge (Saunois et al., 2016).

Methane is primarily formed by degradation of buried organic matter under heat and pressure (thermogenic) inside the earth crust or produced by the incomplete combustion of biomass (pyrogenic). On the other hand, CH_4 resulting from microbial processes, carried out by methanogenic archaea under anoxic conditions in soils and sediments or the digestion system of ruminants are categorized as biogenic or microbial (Kirschke et al., 2013). In contrast to these well-known sources, recent studies have confirmed direct CH_4 release from eukaryotes, including plants, animals, fungi, lichens, and the marine alga *E. huxleyi* even in the absence of methanogenic archaea and in the presence of oxygen or other oxidants (Keppler et al., 2006;Ghyczy et al., 2008;Lenhart et al., 2012;Lenhart et al., 2016;Lenhart et al., 2015b). A very recent study also confirmed *cyanobacteria*, as CH_4 producers suggesting that CH_4 production occurs in all three domains of life (Bizic-Ionescu et al., 2018). These novel sources, from the domains *eucarya* and *bacteria*, might be classified as biotic non-archaeal CH_4 (Boros and Keppler, 2018). In this context, emissions from freshwater and marine cyanobacteria or algae might help to explain the well-known phenomenon of dissolved CH_4 oversaturation in the upper oxic waters of both oceans and lakes that has been often reported from several aquatic environments (Forster et al., 2009;Reeburgh, 2007;Tang et al., 2014;Donis et al., 2017; Bižić-Ionescu et al., 2018;Bange et al., 1994). Significant quantities of CH_4, produced in upper oxic waters, near the air-water interface, might overcome oxidation, and thus significantly contributing to CH_4 fluxes from aquatic environments to the atmosphere (Bogard et al., 2014).

In situ CH_4 production in oxygenated surface waters in the marine environment was first reported by Scranton and Farrington (1977) and Scranton and Brewer (1977) and some decades later also for lakes (Grossart et al., 2011). These results have stimulated the scientific community to study in more detail the phenomenon of CH_4 occurrence in oxygenated surface waters.

For example, it has been suggested that CH_4 might be produced by the bacterial cleavage of methylphosphonate (MPn) in oligotrophic marine pacific waters during phosphorus limitation. (Karl et al., 2008;Metcalf et al., 2012;Repeta et al., 2016). In contrast to this apparently non-oxygen sensitive pathway, many other studies have identified the “traditionally" archaeal methanogenesis in anoxic microenvironments as a CH_4 source. Floating particles (Karl and Tilbrook, 1994), the digestive tracts of zooplankton (de Angelis and Lee, 1994;Stawiarski et al., 2019;Schmale et al., 2018) or fishes (Oremland, 1979) have been found as anoxic micro niches for methanogens. It has been suggested that some methanogens might be active

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under oxic conditions by being equipped with enzymes to counteract the effects of molecular oxygen during methanogenesis (Angel et al., 2011). Potential substrates for methylotrophic methanogens in such micro niches are the algae metabolites dimethylsulfoniopropionate (DMSP) and their degradation products dimethyl sulfide (DMS) or dimethyl sulfoxide (DMSO) (Zindler et al., 2013;Damm et al., 2008;Florez-Leiva et al., 2013). Furthermore, DMSP might also be converted to CH_4 by nitrogen limited bacteria (Damm et al., 2010;Damm et al., 2015). However, in coastal waters where DMS and DMSP production is enhanced, CH_4 was found to mainly related to sedimentary sources (Borges et al., 2018).

In contrast to microbial processes, which are considered to be driven by enzymes, CH_4 might also be derived by the chemical reaction of chromophoric dissolved organic matter (CDOM) and DMS induced by UV or visible light under both oxic and anoxic conditions (Zhang et al., 2015). A similar photochemical CH_4 formation was earlier described for acetone by Bange and Uher (2005) but the production of CH_4 from acetone was considered negligible under oxic conditions.

Another chemical reaction that readily forms CH_4 from the methyl thioethers and their sulphoxides under highly oxidative conditions and catalyzed by nonheme iron-oxo (IV) species was presented by Althoff et al. (2014) and Benzing et al. (2017). Iron-oxo species have been identified as active intermediates in the catalytic cycles of a number of biological enzymatic systems (Hohenberger et al., 2012). Thus, marine algae containing elevated concentrations of methyl thiolethers and their sulfoxides such as DMSP, DMSO, methionine (MET) or methionine sulphoxide (MSO), might be biochemical reactors for non-archaeal CH_4 production as it was already proposed by Lenhart et al. (2016) and Keppler et al. (2009).

Marine phytoplankton plays a central role in the global carbon cycle: Approximately a half of earth's primary production is carried out by marine phytoplankton (Field et al., 1998). So far, direct evidence for CH_4 production by marine algae in the absence of methanogenic archaea has only been provided for *E. huxleyi* (Lenhart et al., 2016). Based on the application of stable carbon isotope techniques, it could be clearly shown that both hydrogen carbonate and a position-specific ^{13}C-labelled MET were carbon precursors of the observed CH_4 production. However, it remains unclear whether CH_4 production also occurs among other marine algae and if there are also other carbon precursors, involved in the formation process.

In the present study we investigated, next to the coccolithophore *E. huxleyi*, two other marine, non-calcifying *haptophy*tes, namely *P. globosa* and *Chrysochromulina sp.* for CH_4 formation. The investigated species are all bloom-forming and often found as dominant members in marine phytoplankton community worldwide (Schoemann et al., 2005;Thomsen, 1994;Brown and Yoder, 1994). Furthermore, they are well-known for their high DMSP, DMS and DMSO productivity (Liss et al., 1994;Keller, 1989;Holligan et al., 1993;Stefels et al., 2007;Matrai and Keller, 1993). We therefore conducted stable isotope experiments using ^{13}C labelled DMS, MSO and DMSO to identify potential methyl group precursor compounds that eventually lead to CH_4 production. Finally, we discuss the laboratory CH_4 production rates in relation to its potential significance in marine environments.

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2. Material & Methods

2.1 Cultures and culture conditions

Three algal species, *Emiliania huxleyi* RCC1216 http://roscoff-culture-collection.org/) *Phaeocystis globosa* PLY 575 and *Chrysochromulina sp.* PLY 307 (https://www.mba.ac.uk/facilities/culture-collection) were studied. All incubation experiments were carried out in controlled and sterile laboratory conditions under a 16/8 hour light/dark cycle at a light intensity of 350 µmol photons m^{-2} s^{-1} and a temperature of 20°C. All samples were taken at the end of the light cycle. Monoclonal cultures were grown in full-batch mode (Langer et al., 2013) in sterile filtered (0.2 µm Ø pore size) in natural North Sea seawater (sampled off Helgoland, Germany) enriched in nutrients according to F/2 medium (Guillard and Ryther, 1962). The initial dissolved inorganic carbon (DIC) of the F/2 medium was 2152 ± 6 µmol L^{-1} (measured by Shimadzu TOC-V CPH).

2.2 Determination of cell densities

Cell densities were determined from four aliquots of each culture sample, using either a Fuschs-Rosenthal or Neubauer counting chamber, depending on cell density.

2.3 Incubation with ^{13}C- labelled hydrogen carbonate

To investigate CH_4 production by algal cultures borosilicate glass bottles (Schott, Germany) filled with 2.0 L 0.2 µm filtered F/2 medium and with 0.35 L headspace volume were used in our investigations of C*hrysochromulina sp.* and *P. globosa*. For the investigations of *E. huxleyi* 0.85 L medium and 0.4 L headspace volume were used (Schott, Germany). The flasks were sealed airtight with lids (GL 45, PP, 2 port, Duran Group) equipped with one three-way port for liquid and a second port fitted with a septum for gas sampling. For measurements of the mixing ratio and stable carbon isotope value of methane ($\delta^{13}C$-CH_4) samples of headspace (20 mL) were taken from each vial. Afterwards, samples (2 mL) for determining cell densities were taken. In order to maintain atmospheric pressure within the vial, the surrounding air was allowed to enter via the three-way port and trough a sterile filter to avoid biological contamination. The inflow of surrounding air was taken into consideration when CH_4 production was calculated.

Main cultures were inoculated from a pre-culture grown in dilute-batch mode (Langer et al., 2009). The initial cell densities were 26.9 ± 4.0 × 10^3 cells mL^{-1} for C*hrysochromulina sp.*, 25.6 ± 1.2 × 10^3 cells mL^{-1} for *P. globosa* and 17.5 ± 2.0 × 10^3 cells mL^{-1} for *E. huxleyi*. headspace and liquid samples were collected on a daily basis for *E. huxleyi* and in 2-3 days intervals from cultures of *Chrysochromulina sp.* and *P. globosa*. The overall sampling time was 9, 11 and 6 days with final

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cell densities of 0.18 ± 0.01 × 10^6 cells mL^{-1}, 1.77 ± 0.15 × 10^6 cells mL^{-1} and 1.70 ± 0.09 × 10^6 cells mL^{-1} for *Chrysochromulina sp.*, *P. globosa* and *E. huxleyi* respectively.

Cell densities were plotted versus time and the exponential growth rate (μ) was calculated from exponential regression using the natural logarithm (Langer et al., 2013). The phase of exponential growth (from which μ was calculated) was defined by the cell densities which corresponded to the best fit ($r^2 > 0.99$) of the exponential regression. This was done by using the first three (*Chrysochromulina sp.* and *E. huxleyi*) or four data points (*P. globosa*) of the growth curve.

Four different treatments were used: medium either with (medium + HCO_3^-) or without (medium) a treatment of $H^{13}CO_3^-$ and cultures supplemented either with (culture + HCO_3^-) or without (culture) $H^{13}CO_3^-$ (n=3). Please note that stable isotope measurements using $H^{13}CO_3^-$ were not performed for *E. huxleyi* as evidence for isotope labelling of CH_4 formation was already provided by Lenhart et al. (2016).

For stable carbon isotope experiments 48,7 μmol L^{-1} ^{13}C-hydrogen carbonate ($NaH^{13}CO_3$) in final concentration was added to the F/2 medium resulting in a theoretical calculated ^{13}C value of DIC of +2014 ± 331‰. To determine the $\delta^{13}C$-CH_4 values of the source, the Keeling-plot method was applied (Keeling, 1958). For a detailed discussion of the Keeling plot method for determination of the isotope ratio of CH_4 in environmental applications, please refer to (Keppler et al., 2016). Oxygen concentration was monitored daily (using inline oxygen sensor probes, PreSens, Regensburg) at the end of the light cycle (S1).

2.4 Determination of CH_4 production rates

Since the experiment in the section 3.2 was not designed to obtain POC quotas (POC = particulate organic carbon), we conducted an additional experiment. To best compare CH_4 formation rates of the three algae species it is necessary to obtain exponential growth to ensure constant growth rates and constant cellular POC quotas over the course of the experiment (Langer et al., 2012, 2013). The CH_4 production rates can be calculated by multiplying the growth rate μ with the corresponding cellular or POC-CH_4 quota, that was measured on the end of the experiment.

The cultures were grown in 160 ml crimped serum bottles filled with 140 ml medium and 20 ml headspace. The initial cell density of 22.5 ± 3.1 × 10^3 cells mL^{-1}, 80.9 ± 11.5 × 10^3 cells mL^{-1} and 29.0 ± 5.5× 10^3 cells mL^{-1} for *Chrysochromulina sp.*, *P. globosa* and *E. huxleyi*, respectively were inoculated. Cultures were gown up to 37.0 ± 9.2 × 10^3 cells mL^{-1} (*Chrysochromulina sp.*), 219 ± 24.1 × 10^3 cells mL^{-1} (*P. globosa*) and 283 ± 15.6 × 10^3 cells mL^{-1} (*E. huxleyi*). These cell densities corresponded to the cell densities of exponential growth phase obtained from the experiment in section 3.2. Oxygen concentration was monitored (using inline oxygen sensor probes, PreSens, Regensburg) at the end of each light and dark cycle (S2).

The growth rate (μ) was calculated from cell densities of the beginning and end of the experiment according to Eq.1:

$$\mu = \frac{Ln(N_1) - Ln(N_0)}{(t_1 - t_0)} \quad (1)$$

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where N_0 and N_1 are the cell densities at the beginning (t_0) and end of the experiment (t_1). The daily cellular CH_4 production rates (CH_4P_{cell}, ag CH_4 cell^{-1} d^{-1}, ag = 10^{-18} g) were calculated according to Eq.2:

$$CH_4P_{cell} = \mu \times \frac{m(CH_4)}{cell} \quad (2)$$

where $m(CH_4)$ is the amount of CH_4 that was produced at the end of the experiment.

To calculate POC based CH_4 production rates the cellular organic carbon content (POC_{cell}) was derived from cell volume (V_{cell}) by using the Eq. 3 according to (Menden-Deuer and Lessard, 2000):

$$POC_{cell} = 0.216 \times {V_{Cell}}^{0.939} \quad (3)$$

The cell volume was determined measuring the cell diameter in light micrographs using the program Image J. According to (Olenina, 2006) a ball shape can be assumed for calculating the cell volume for the three species investigated here. The daily cellular CH_4 production rates (CH_4P_{POC}, µg CH_4 g^{-1} POC d^{-1}) were calculated from growth rate and CH_4-POC quotas at the end of the experiment according to Eq. 4.

$$CH_4P_{POC} = \mu \times \frac{m(CH_4)}{POC} \quad (4)$$

The CH_4 production potential (CH_4-PP) was used to translate differences in cellular production rates to community level. According to (Gafar et al., 2018), the CH_4-PP can be calculated for different periods of growth, by calculating a cellular standing stock for each time period from a known starting cell density (N_0) (whereby constant exponential growth is assumed). The corresponding amount of produced CH_4 (CH_4PP) for each period of growth and standing stock is the product of the cellular standing stock and CH_4 quota (Eq. 5).

$$CH_4PP = N_0 \times e^{\mu \times t} \times \frac{m(CH_4)}{cell} \quad (5)$$

In the present study the CH_4-PP was calculated for a standing stock that is obtained after 7 days of growth and starting by a cell count of one single cell.

2.5 Incubation with ^{13}C labelled DMS, DMSO and MSO

The sulphur bonded methyl group(s) in DMS, DMSO and MSO were investigated as precursors for algal-derived CH_4 in an incubation experiment with *E. huxleyi*. For all tested compounds only the C atom of the sulphur bonded methyl group(s) was labelled with ^{13}C (R-S-$^{13}CH_3$, 99%). A final concentration of 10 µM were used for each compound. The treatments were initiated in parallel from batch culture by inoculating 17.5 ± 2.0 × 10^3 cells mL^{-1} and cultures were grown to final cell densities of 1.77 ± 0.08 × 10^6 cells mL^{-1}. For every single compound ($^{13}C_2$-DMS, $^{13}C_2$-DMSO,^{13}C-MSO) four treatment groups with three independent replicates and repeated measurements over time were used analogous to experiments

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described in section 3.2 for *E. huxleyi*. With reference to section 3.2 the experiment differs in sampling frequencies and overall incubation time: samples were taken daily during an overall incubation time of 6 days.

2.6 Determination of CH_4 mass

Five mL of a gas sample was collected from the head space of the vials using a gas tight Hamilton gas syringe. The sample was analyzed by gas chromatography (GC-14B, Shimadzu, Japan; column: 2 m, Ø = 3.175 mm inner diameter, high-grade steel tube packed with Molecular Sieve 5A 60/80 mesh from Supelco) equipped with a flame ionization detector (FID). Quantification of CH_4 was carried out by comparison of the integrals of the peaks eluting at the same retention time as that of the CH_4 authentic standard, using two reference standards containing 9837 and 2192 parts per billion by volume (p.p.b.v). Mixing ratios were corrected for head space pressure that was monitored using a pressure measuring device (GMSD 1,3 BA, Greisinger).

The CH_4 mass (m_{CH_4}) was determined by its mixing ratio (x_{CH_4}) and the ideal gas law (Eq 6),

$$m_{CH_4} = M_{CH_4} \times x_{CH_4} \frac{p \times V}{R \times T} \quad (6)$$

where M_{CH_4}= molar mass, p = pressure, T = temperature, R = ideal gas constant, V = volume.

2.7 GC-C-IRMS measurements

Stable carbon isotope values of CH_4 of headspace samples were analysed by gas chromatography stable isotope ratio mass spectrometry (GC-C-IRMS, Deltaplus XL, Thermo Finnigan, Bremen, Germany). All δ^{13}C-CH_4 values were corrected using two CH_4 working standards (isometric instruments, Victoria, Canada) with values of -23.9 ± 0.2‰ and -54.5 ± 0.2‰. The results were normalized by two-scale anchor calibration according to (Paul et al., 2007). The average standard deviation of the analytical measurements was in the range of 0.1 ‰ to 0.3 ‰ (three repeated measurements of CH_4 working standards). All δ^{13}C-CH_4 values are expressed in the conventional δ notation, in per mille (‰) vs. Vienna Pee Dee Belemnite (VPDB), using Eq.7.

$$\delta^{13}C = \frac{\left(\frac{^{13}C}{^{12}C}\right)_{sample}}{\left(\frac{^{13}C}{^{12}C}\right)_{standard}} - 1 \quad (7)$$

For a detailed description of the δ^{13}C-CH_4 measurements by GC-IRMS and technical details of the pre-concentration system we would like to refer to previous studies by (Comba et al., 2018) and (Laukenmann et al., 2010)

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2.8 Statistics

To test for significant differences in cell density, CH_4 formation, and CH_4 content between the treatments, two-way analysis of variance (ANOVA) (considering repeated measurements) and a post hoc test [Fisher least significant difference (LSD) test; alpha 5 %] were used.

3. Results

3.1 Algal growth and CH_4 formation

The growth curves during incubation of the three algal species at a temperature of 20°C and a day-night-cycle of 16 h day 8 h night are presented in Fig. 1 (upper panel a, b, c). The initial cell densities were 26.9 ± 4.0 × 10^3 cells mL^{-1} for C*hrysochromulina sp.*, 25.6 ± 1.2 × 10^3 cells mL^{-1} for *P. globosa* and 17.5 ± 2.0 × 10^3 cells mL^{-1} for *E. huxleyi*. The exponential growth rate μ was highest for *E. huxleyi* (1.71 ± 0.04 d^{-1}) i.e. three or five times higher than for *P. globosa* and C*hrysochromulina sp.* (with 0.33 ± 0.08 d^{-1} and 0.52 ± 0.07 d^{-1}, respectively). These rates were obtained by exponential regression from the first three (*Chrysochromulina sp.* and *E. huxleyi*) or four time points (*P. globosa*) of the growth curve, while cell densities on the following timepoints departs from the one expected from exponential growth (dotted line, Fig. 1 a, b, c,). Maximum cell densities were lowest for C*hrysochromulina sp.* with 0.18 ± 0.01 × 10^6 cells mL^{-1} followed by *E. huxleyi* with 1.70 ± 0.09 × 10^6 cells mL^{-1} and highest for *P. globosa* with 1.77 ± 0.15 × 10^6 cells mL^{-1}.

Significant CH_4 formation was observed in all three cultures over the whole incubation period of 5 to 11 days (Fig 1 d, e, f) whereas no increase in CH_4 over time was observed in the control groups. For all species the increase in headspace CH_4 was significant ($p \leq 0.05$) at second time point of measurement and at all following time points ($p \leq 0.001$). At the end of the incubation period the amounts of produced CH_4 were 30.0 ± 6.2 ng, 85.2 ± 10.0 ng and 43.5 ± 4.3 ng for *Chrysohromulina. sp.*, *P. globosa* and *E. huxleyi*, respectively. A linear correlation was found between the absolute number of cells and the amount of produced CH_4 of *Chrysochromulina sp.*, *P. globosa* and *E. huxleyi* (Fig. 1 lower panel, g, h, i).

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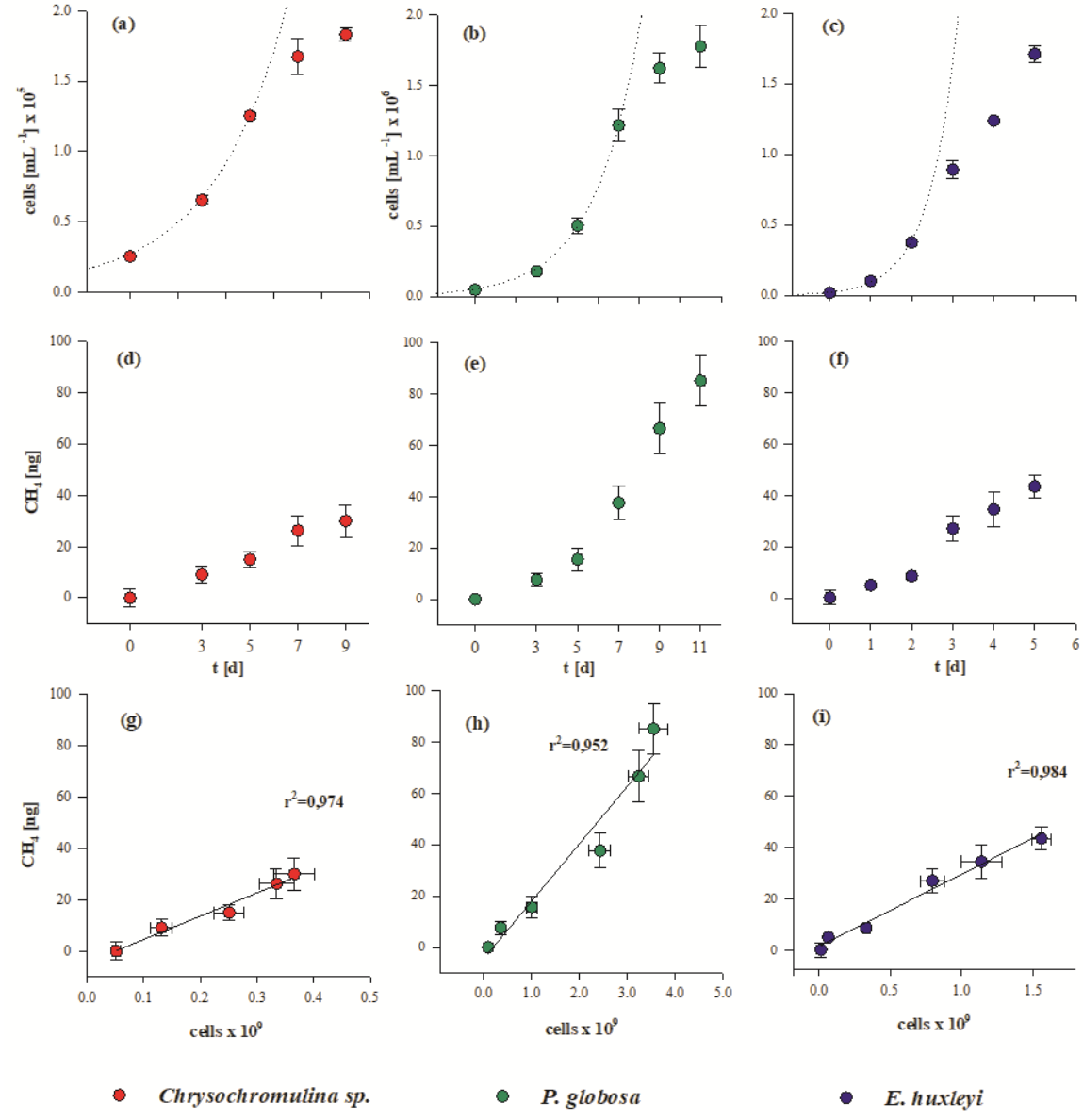

220 **Fig. 1: Cell growths (first panel), CH_4 production (middle panel) in course of time and correlation between the total number of cells and produced CH_4 (lower panel) from three algae species. *Chrysochromulina sp.* (left column a, d, g,), *P. globosa* (middle column b, e, h) and from *E. huxleyi* (right column c, f ,i). Please note that the cell numbers of *Chrysochromulina sp.* are presented in 10^5 and P. *globosa*, *E. huxleyi* in 10^6. Mean values of six (*Chrysochromulina sp.*, *P. globosa*) and three (*E. huxleyi*) replicated culture experiments are shown and error bars mark the SD.**

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3.2 Stable carbon isotope values of CH_4 during incubation with ^{13}C-hydrogen carbonate

Stable carbon isotope values of CH_4 ($\delta^{13}CH_4$ values) for *Chrysochromulina sp.* and *P. globosa* are presented in Fig. 2 (a, c). We observed conversion of ^{13}C carbon (provided by ^{13}C-hydrogen carbonate) to $^{13}CH_4$ in cultures of both species, indicated by increasing $\delta^{13}CH_4$ values over time. Stable isotope values increased from initial atmospheric (laboratory air) levels of -48.7 ± 0.3 ‰ and -48.4 ± 0.10 ‰ up to +30.1 ± 10.2 ‰ and +245 ± 16 ‰ for *Chrysochromulina sp.* and *P. globosa*, respectively, whilst the $\delta^{13}CH_4$ values of the control groups (algae without ^{13}C-hydrogen carbonate or ^{13}C-hydrogen carbonate in medium without culture) did not change over time. The increase of $\delta^{13}CH_4$ values in the headspace-CH_4 depended on the amount of released CH_4 that was added to the initial (atmospheric) background level. To calculate the $\delta^{13}CH_4$ values of the CH_4 source which has raised CH_4 quantity above background level the Keeling-plot method (Keeling, 1958;Pataki et al., 2003) was used (Fig. 2 b, d).

The calculated $\delta^{13}CH_4$ values of the CH_4 source were +1300 ± 245 ‰ (*Chrysochromulina sp.*) and +1511 ± 35 ‰ (*P. globosa*) and thus close to the theoretical calculated ^{13}C value of the DIC (2014 ± 331‰) resulting from the addition of ^{13}C-hydrogen carbonate. Please note that ^{13}C-hydrogen carbonate stable isotope labelling experiment with *E. huxleyi* were already performed by Lenhart et al. (2016) and were not repeated in this study. This is why $\delta^{13}CH_4$ values and the respective Keeling plot of *E. huxleyi* are not shown in Fig. 2.

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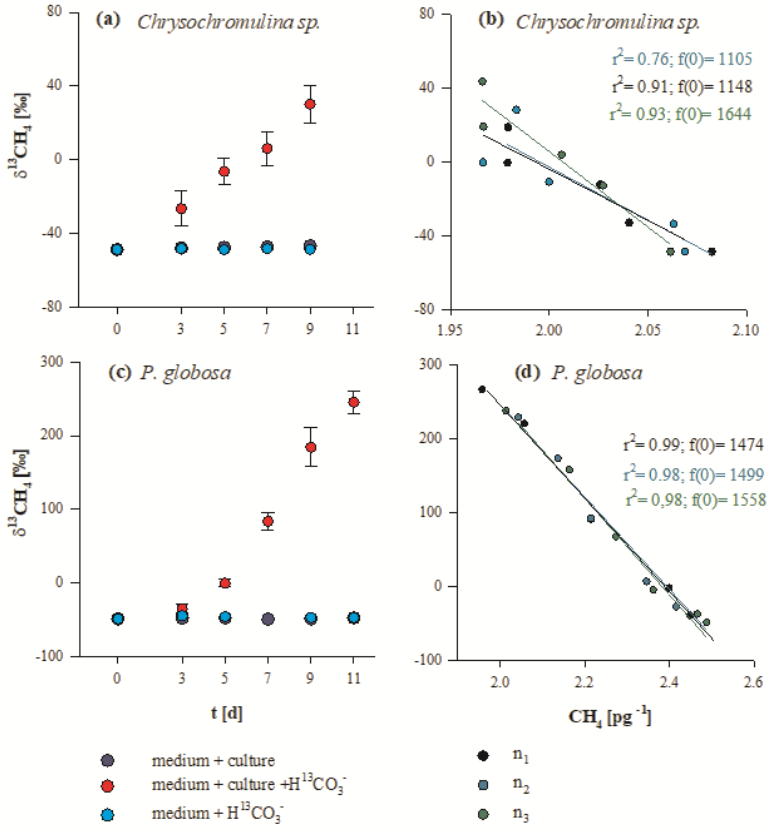

Fig. 2: $\delta^{13}CH_4$ values (left column) and respective Keeling plots (right column) from *Chrysochromulina sp.* (a,b) and *P. globosa* (c,d) after the addition of $H^{13}CO_3^-$. The left column (a, c) shows the $\delta^{13}CH_4$ values of three investigation groups ("culture + $H^{13}CO_3^-$ ", "culture" and "$H^{13}CO_3^-$"), whereas each data point presented is the mean value of three replicated culture experiments with error bars showing SD. The right column shows the Keeling plots for the treatments "culture + $H^{13}CO_3^-$" from each replicated culture experiments (n_1, n_2, n_1) where f (0) refers to the ^{13}C value of the CH_4 source.

3.3 CH_4 production and production potential

To estimate CH_4 production rates of the three algal species it is necessary to ensure exponential growth. We normalized CH_4 production rates to cell and to particulate organic carbon (POC) content. By doing so the CH_4 production rate is the product of exponential growth rate µ and cellular or POC quota. Since the experiment in the section above (isotope measurements) was not designed to obtain POC quotas, we conducted an additional experiment.

The POC normalized daily CH_4 production rate was highest in *E. huxleyi*, followed by *P. globosa*, and *Chrysochromulina sp.*. However, the cellular or POC normalized daily production rates of the three algal species were in the same order of

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magnitude (Tab. 2). We calculated the CH_4 production potential (CH_4PP), that is the amount of CH_4 produced within a week of growth (Gafar et al., 2018), to translate the cellular production rates ($\mu \times CH_4$ cell^{-1}) of each species to community level. The CH_4PP was two order of magnitude higher for *E. huxleyi* than the other two species. This is a consequence of the higher growth rate of *E. huxleyi*.

We furthermore observed the oxygen concentrations during the light and dark periods to ensure oxic conditions. The measured oxygen concentrations were always saturated or supersaturated (S2).

Tab 1: Growth rate, cellular POC, CH_4 production rates and $CH_4PP_{(7days)}$ of *Chrysochromulina sp.* (n=4), *P. globosa* (n=4) and *E. huxleyi* (n=4). Values are the mean of four replicated culture experiments with SD.

	growth rate (μ)	cellular POC	CH_4 production rate		$CH_4PP_{(7days)}$
	d^{-1}	pg cell^{-1}	ag CH_4 cell^{-1} d^{-1}	μg CH_4 g^{-1} POC d^{-1}	fg CH_4
Chrysochromulina sp.	0.21 ± 0.04	25.4 ± 4.0	38.9 ± 10.6	1.6 ± 0.5	0.8 ± 0.3
P. globosa	0.50 ± 0.06	7.0 ± 0.4	14.4 ± 5.6	2.1 ± 0.8	1.0 ± 0.3
E. huxleyi	1.09 ± 0.02	20.1 ± 0.7	53.3 ± 5.5	2.7 ± 0.7	104 ± 8.0

3.4 CH_4 formation from ^{13}C labelled methyl thiol ethers

The three methylated sulphur compounds MSO, DMSO and DMS were tested for potential CH_4 formation in incubation experiments with *E. huxleyi*. For all tested compounds ^{13}C isotope labelling technics where applied where only the methyl bonded sulphur group(s) (R-S-$^{13}CH_3$) was fully labelled with ^{13}C atoms. Cell densities and CH_4 formation correlated in all treatments, while no difference in cell growth pattern or CH_4 formation was observed when isotope labelled methyl thioether and sulfoxides were added to the culture (Fig. 3 a, b, c). Differences between treatments were found in $\delta^{13}CH_4$ values of headspace CH_4. The initial $\delta^{13}CH_4$ value of headspace (-47.9 ± 0.1 ‰, laboratory air) increased slightly over time in untreated cultures (without isotope treatment) to -46.8 ± 0.3 ‰ (Fig 4.b).

In contrast, experiments where $^{13}C_2$-DMSO, $^{13}C_2$-DMS and ^{13}C-MSO was applied to cultures of *E. huxleyi* $\delta^{13}CH_4$ values increased to -31.0 ± 1.1 ‰, -45.7 ± 0.1 ‰ and +18.3 ± 7.7 ‰, respectively over a time period of 6 days (Fig. 4 a, b, c) and differed significantly from control groups ($p<0.05$).

The results unambiguously show that a fraction of the ^{13}C-labelled methyl groups of the added substances was converted to ^{13}C-CH_4 in cultures of *E. huxleyi*. Much smaller changes in $\delta^{13}CH_4$ values were observed for controls of sterile filtered media where only $^{13}C_2$-DMS and ^{13}C-MSO was added (-42.8 ± 1.7 ‰ and -43.9 ± 0.2 ‰ respectively, Fig. 4 a, c, day 6), whereas $\delta^{13}CH_4$ values did not change over time in the seawater controls (no addition of isotopic labelled compounds) and in the seawater controls treated with $^{13}C_2$-DMSO (Fig. 4 b). Based on the initial amount of ^{13}C label substance that were added to

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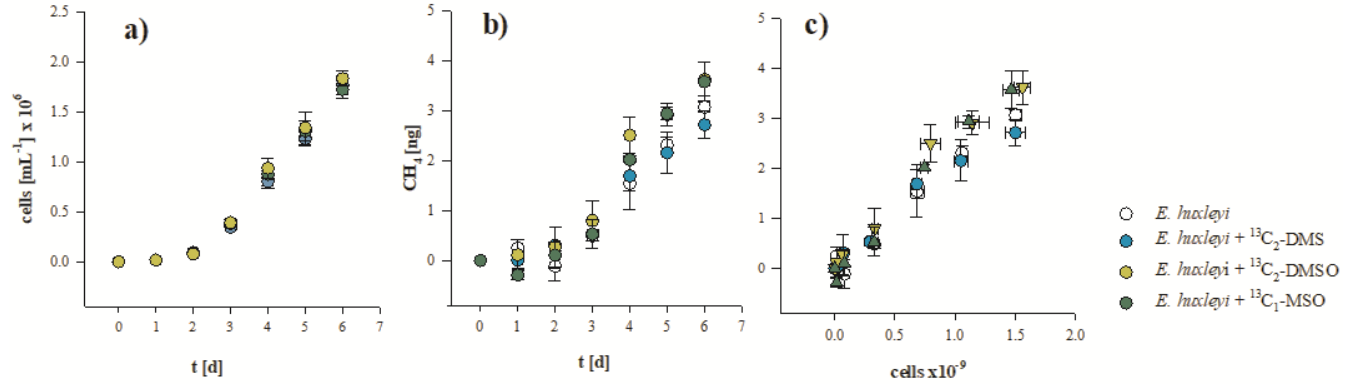

Fig. 3 Cell growths (a), CH_4 production (b) and relation between the total number of cells and produced CH_4 (c) from *E. huxleyi* treated with $^{13}C_2$-DMS, $^{13}C_2$-DMSO and ^{13}C-MSO or without any treatment. Mean values of three replicated culture experiments are shown and error bars mark the SD.

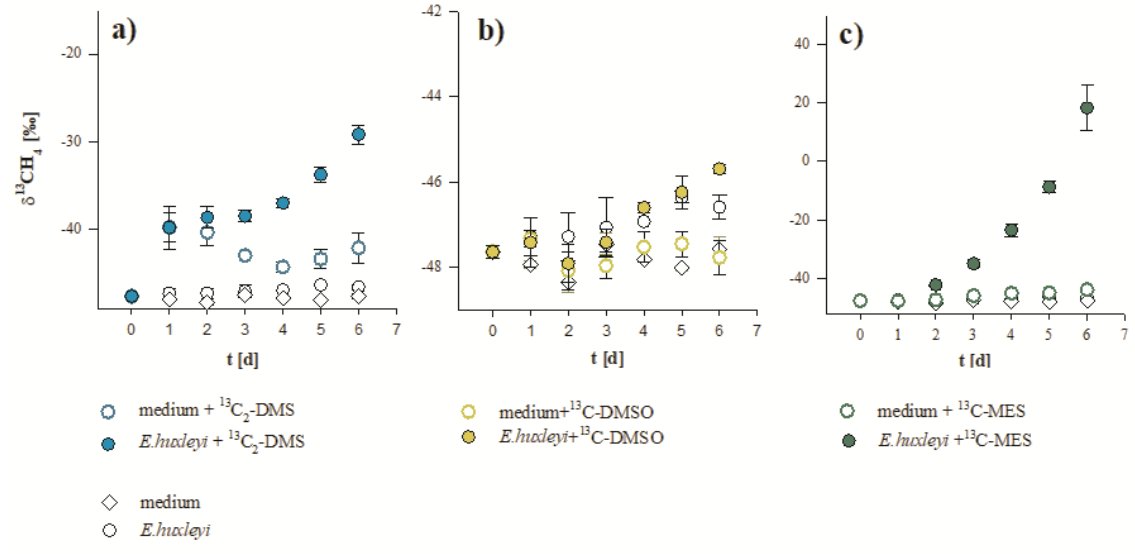

Fig 4. $^{13}CH_4$ values of headspace CH_4 in cultures of *E. huxleyi* supplemented with (a) $^{13}C_2$-DMS, (b) $^{13}C_2$-DMSO and (c) ^{13}C-MSO. Mean values of three replicated culture experiments are shown and error bars mark the SD.

4. Discussion

Our results of CH_4 production and stable carbon isotope measurements provide unambiguous evidence that next to *E. huxleyi* (Lenhart et al., 2016) other widespread marine algal species namely *Chrysochromulina sp.* and *P. globosa* produce CH_4 per

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se under oxic conditions at rates of 1.6 ± 0.5 to 2.7 ± 0.7 µg CH_4 g^{-1} POC d^{-1}. The three investigated genera of marine phytoplankton have a world-wide distribution and they are representatives of the most widespread marine haptophytes (Schoemann et al., 2005;Thomsen, 1994;Brown and Yoder, 1994). The results indicate that CH_4 production could be a common process across marine haptophytes. We first discuss the stable isotopic evidence of CH_4 formation, the role of precursor compounds and likely mechanisms involved. Finally, we discuss the laboratory CH_4 production rates in relation to its potential significance in marine environments and provide a first rough estimation how these production rates might contribute to CH_4 concentration in oxic surface waters previously reported in open ocean algal blooms.

In cultures of *Chrysochromulina sp*. and *P. globosa*, that were treated with ^{13}C-labelled hydrogen carbonate, $\delta^{13}CH_4$ values increased with incubation time, clearly resulting from the conversion of ^{13}C-hydrogen carbonate to $^{13}CH_4$. These results demonstrate that all three investigated algal species produce CH_4 per se under oxic conditions (S.1) and that hydrogen carbonate serves as a carbon source for $^{13}CH_4$. Our findings are in agreement with the stable isotope evidence of CH_4 production by *E. huxleyi* (Lenhart et al., 2016). However, we do not consider hydrogen carbonate as the direct carbon precursor of CH_4. In a first step hydrogen carbonate and its isotope label is converted to CO_2 and subsequently fixed by algal primary production forming POC. Therefore, we would expect a large fraction of the ^{13}C label of the hydrogen carbonate (+2014 ± 331‰) to be transferred to the POC towards the end of the experiment (with highest cell numbers). The experiments were started by inoculation cells from pre-cultures, that were grown on DIC with natural $^{13}C/^{12}C$ abundance (δ^{13}C values ~0 ‰). This means that during ongoing incubation the δ^{13}C-POC value should get close to δ^{13}C-DIC values, resulting from the addition of ^{13}C-hydrogen carbonate, when cultures grow in the new ^{13}C enriched medium. Consequently, the δ^{13}C-POC values are considered to be somewhat lower than the theoretically calculated δ^{13}C-DIC values (+2014 ± 331‰) of the medium. Our assumptions are in line with the $\delta^{13}CH_4$ source signature values (averaged over 9 or 11 days respectively), obtained via Keeling plot method, which were +1300 ± 245 ‰ and +1511 ± 35 ‰ for *Chrysochromulina. sp*. and *P. globosa*, respectively and thus were somewhat lower than for the theoretical calculated ^{13}C value of the DIC (+2014 ± 331‰) resulting from the addition of ^{13}C-hydrogen carbonate. Unfortunately, δ^{13}C-DIC and δ^{13}C-POC values could not be determined in our set of experiments to allow more detailed calculations. However, our results clearly indicate that hydrogen carbonate is the principle inorganic carbon precursor of $^{13}CH_4$ produced in algae, but intermediate metabolites are likely to be formed from which CH_4 is released, possibly by cleavage of sulphur-bonded methyl groups of methyl thioethers and sulfoxides (Althoff et al., 2014, Lenhart et al., 2016, Benzing et al., 2017).

4.1 CH_4 formation from ^{13}C labelled methyl thioethers

Methyl thioethers are precursors of CH_4

Methyl thioethers and their sulphoxides are ubiquitous in marine environments as they are often produced by algae at substantial rates. It is also known that these compounds are metabolized in the three investigated algal species (Liss et al., 1994;Keller, 1989). Based on the addition of $^{13}C_2$-DMSO, $^{13}C_2$-DMS and ^{13}C-MSO, where only the sulphur-bonded methyl

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groups (–S-CH_3) were 99% labelled with ^{13}C, it was possible to clearly monitor $^{13}CH_4$ formation by stable carbon isotope measurements in cultures of *E. huxleyi*. The $\delta^{13}CH_4$ values, increased over time significantly in $^{13}C_2$-DMS, $^{13}C_2$-DMSO and ^{13}C-MSO treated cultures, above the $\delta^{13}CH_4$ values of the control groups. (Fig.4 a-c). The $^{13}CH_4$ quantity from conversion of ^{13}C labelled substance explains roughly 0.03% ($^{13}C_2$-DMSO) up to 0.84% (^{13}C-MSO) to overall released CH_4. However, even if the biggest fraction of CH_4 in algae cultures was not released by the ^{13}C labelled substances, the delta notation values in cultures demonstrate that ^{13}C labelled precursor substances were converted to CH_4 by the algae. These isotope labelling results are also in good agreement with recent results from laboratory experiments where MET was added to cultures of *E. huxleyi* (Lenhart et al., 2016). In addition, we also found an indication for a purely chemical CH_4 formation pathway from control samples using sterile seawater and addition of either $^{13}C_2$-DMS and ^{13}C-MSO. A similar observation was already made by Lenhart et al. (2016) when applying ^{13}C-MET in seawater. However, the relatively slight increase in $\delta^{13}CH_4$ values in the control samples (Fig.4a) implicates that this is only a minor pathway. The CH_4 conversion from ^{13}C-DMS and ^{13}C-MSO in seawater was approximately 3- and 30-fold lower than in the corresponding treatments with algae and becomes only obvious when applying stable isotope labelling experiments. However, this observation might be in agreement with previously findings by Zhang et al. (2015), who described a photochemically and CDOM related conversion of DMS to CH_4 in oxygenated natural seawater.

Reasons for variable conversion of methyl thioethers to CH_4

The conversion of the initial added amount of ^{13}C-labelled substance (8,5 µmol) into $^{13}CH_4$ varied drastically between supplemented compounds in algal cultures ranging from 3.0 pmol ($^{13}C_2$-DMSO), 9.5 pmol ($^{13}C_2$-DMS), to 30.1 pmol (^{13}C-MSO). However, these conversion quantities of ^{13}C-labelled substance in algal cultures were about nine ($^{13}C_2$-DMS), three ($^{13}C_2$-DMSO) and thirty (^{13}C-MSO) times higher than in control groups. Several explanations for variable conversion of methyl thioethers to CH_4 can be given: firstly newly synthesized, non-labelled molecules of the applied ^{13}C substances are produced by the cells to a different extent, which in turn leads to different cell concentrations of ^{13}C labelled molecules (due to the relative depletion of ^{13}C labelled substances). Secondly, the applied compounds penetrate the cells to a different extent leading to different final concentrations inside the cells. For instance, amino acids like MET and MSO are taken up by transporter systems (Cho and Komor, 1985) while DMS is much more hydrophobic than DMSO and is highly permeable to cell membranes (Sunda et al., 2002;Spiese et al., 2015) . Finally, the reactivity of the methyl bonded sulphur group of the methyl thioethers and their sulfoxides differs between the chemical compounds. For instance, the CH_4 conversion were variable between methylated sulphur compounds, when Althoff et al. (2014) used different methylated sulphur compounds to mimic CH_4 formation in eukaryotes via chemical reaction.

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Mechanism of CH_4 formation from thioethers

The CH_4 formation from thioethers (MET, DMS) and their corresponding sulphoxides (MSO, DMSO) might be catalysed by nonheme oxo iron (IV), thus forming methyl radicals ($\cdot CH_3$) from homolytically broken sulphur methyl bounds ($R\text{-}CH_3$) leading to CH_4 under oxidative conditions (Althoff et al., 2014, Benzing et al. 2017). Since the tested compounds are found in high cellular concentrations in *E. huxleyi*, *Chrysochromulina sp.* and *P. globosa* and non heme oxo iron (IV) have been identified as active intermediates in the catalytic cycles of a number of biological enzymatic systems (Hohenberger et al., 2012), the postulated reaction might be a likely pathway for CH_4 production in investigated alga species. Furthermore, DMS and DMSO were described to be part of an antioxidant system as these compounds can readily scavenge hydroxyl radicals in cells of E. huxleyi (Sunda et al., 2002). Furthermore, CH_4 is released via a methyl radical, that is subtracted from DMSO when hydroxyl radicals being scavenged – and accordingly DMS after its sulphoxidation (Herscu-Kluska et al., 2008). Since MET and MSO have similar functional groups to DMS and DMSO respectively, it was proposed that the reaction described above is taking place analogously for these compounds (Bruhn et al., 2012;Lenhart et al., 2015a). Consequently, the CH_4 formation in investigated algal species might be a response of oxidative stress, that forms hydroxyl radicals or other reactive oxygen species (ROS), which in turn might react with the applied methylated sulphur compounds generating methyl radicals and eventually CH_4.

Methyl thioethers are not converted by methanogenic archaea in our cultures

The algal metabolites DMSP, DMS and DMSO are ubiquitous in marine surface layers and nanomolar concentrations were found in blooms of *Chrysochromulina sp*., *P. globosa* and *E. huxleyi*. Several field studies showed that these compounds are linked to CH_4 formation in seawater (Zindler et al., 2013;Damm et al., 2008;Florez-Leiva et al., 2013). The authors proposed that DMSP and their degradation products DMSO and DMS are used by methylotrophic methanogenic archaea, inhabiting in anoxic microsites, as substrates for methanogenesis. While methanogenesis in anoxic microsites is a proposed source in the field, an involvement of methanogenic archaea is highly unlikely in our culture experiments because experiments were performed under sterile and oxic conditions. High oxygen accumulation due to algal photosynthesis was observed in all experiments (S1, S2). Furthermore, the absence of methanogenic archaea in culture experiments of *E. huxleyi* strain RCC1216 using several molecular tools was clearly shown in a previous study (Lenhart et al., 2016).

4.2 POC normalized production

For all three algal species significant correlations between CH_4 mass and cell density was found ($r^2 > 0.95$ for all species, Fig 1 g, h, i), suggesting that CH_4 formation occurred over the entire growth curve.

However, since CH_4 production can only be determined in the exponential phase (Langer et al., 2013) we additionally ran dilute batch cultures to determine CH_4 production. All three species displayed similar CH_4 production ranging from 1.6 ± 0.5

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to 2.7 ± 0.7 µg CH_4 g^{-1} POC d^{-1} with *Chrysochromulina sp.* and *E. huxleyi* showing the lowest and highest rates, respectively. The CH_4 production for *E. huxleyi* was found to be twofold higher than rates reported for the same strain and comparable culture conditions by Lenhart et al. (2016) (0.7 µg CH_4 g^{-1} POC d^{-1}). The lower production reported by Lenhart et al. (2016) may be explained by the fact that CH_4 production was not obtained from exponentially growing cultures.

However, we do suggest that CH_4 production of various algae might differ substantially under changing environmental conditions, as already shown for terrestrial plants (Abdulmajeed and Qaderi, 2017;Martel and Qaderi, 2017). Moreover, the cellular concentrations of potential precursor compounds such as methylated sulphur compounds might vary greatly between species and cultures. The investigated algal species can reach millimolar intracellular concentrations of DMS and DMSP (Sunda et al., 2002; Liss et al., 1994;Keller, 1989) and even if the conversion rate of methylated sulphur compounds to CH_4 in algal cells might be low, they could be sufficient to explain a substantial fraction of the CH_4 production rates by marine algae.

4.3 Implication for the marine environment and algal blooms

It has been hypothesized that CH_4 formation in oxic surface waters is associated with primary production since a correlation between chlorophyll a and dissolved CH_4 was found, and CH_4 saturation increased during algal blooms in both lakes and oceans (Weller et al., 2013).

It was estimated that the gross CH_4 production in a southwest Pacific Ocean mesoscale eddy is 40 - 58 pmol CH_4 L^{-1} d^{-1} (Weller et al., 2013). Using reported phytoplankton cell densities (1.7×10^8 to 2.9×10^8 cells L^{-1}, Weller et al., 2013), we calculated a maximal cellular production of 5.5 ag CH_4 $cell^{-1}$ d^{-1} for this eddy. The species investigated in this study showed ca. 3-9 times higher cellular production (Table 1). Hence each of the three haptophyte algae studied here could account for the CH_4 production reported by Weller et al. (2013). Judging from cellular production, the species studied here are of similar importance for oceanic CH_4 production in biogeochemical terms. Regarding the highest cellular production, that of *E. huxleyi* as 100%, *P. globosa* produces 27% and *Chrysochromulina sp.* 87% (Table 1). However, while cellular production is an interesting parameter in physiological terms, it is not useful in biogeochemical terms. Gafar et al. (2018) suggested the production potential (PP), as opposed to cellular production, as a biogeochemically meaningful parameter. We calculated the CH_4-PP (Material and Methods) for our three species, and when the one of *E. huxleyi* is considered 100%, *P. globosa* has a CH_4-PP of 1%, and *Chrysochromulina sp.* 0.8% (Table 1). In terms of CH_4 production in the field, therefore, *E. huxleyi* out-performs the other two haptophytes by two orders of magnitude. It can be concluded that the CH_4-PP under given environmental conditions is species-specific and therefore community composition will have an influence on algal sea surface water CH_4 production.

It can be hypothesized that changing environmental conditions might drastically affect algal CH_4 production, which has to be taken into account when calculating annual averages. The effect of dominant environmental parameters such as light intensity and temperature on algal CH_4 production will therefore be the subject matter of future studies.

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Competing interests: The authors declare that they have no conflict of interest.

Acknowledgment: We thank Markus Greule, Bernd Knape and Stefan Rheinberger for conducting analytical measurement and technical support that helped to produce this dataset. F.K. and T.K. were supported by the German Research Foundation
425 (DFG; KE 884/8-2, KE 884/11-1 and KE 884/16-2), G.L. by the Natural Environment Research Council (NE/N011708/1).

https://doi.org/10.5194/bg-2019-245
Preprint. Discussion started: 1 July 2019

References

Abdulmajeed, A. M., and Qaderi, M. M.: Intrashoot variation in aerobic methane emissions from pea plants exposed to multiple abiotic stresses, Acta Physiologiae Plantarum, 39, 124, 2017.

Althoff, F., Benzing, K., Comba, P., McRoberts, C., Boyd, D. R., Greiner, S., and Keppler, F.: Abiotic methanogenesis from organosulphur compounds under ambient conditions, Nat Commun, 5, 10.1038/ncomms5205, 2014.

Angel, R., Matthies, D., and Conrad, R.: Activation of Methanogenesis in Arid Biological Soil Crusts Despite the Presence of Oxygen, Plos One, 6, 10.1371/journal.pone.0020453, 2011.

Bange, H. W., Bartell, U., Rapsomanikis, S., and Andreae, M. O.: Methane in the Baltic and North Seas and a reassessment of the marine emissions of methane, Global biogeochemical cycles, 8, 465-480, 1994.

Bange, H. W., and Uher, G.: Photochemical production of methane in natural waters: implications for its present and past oceanic source, Chemosphere, 58, 177-183, 10.1016/j.chemosphere.2004.06.022, 2005.

Benzing, K., Comba, P., Martin, B., Pokrandt, B., and Keppler, F.: Nonheme Iron-Oxo-Catalyzed Methane Formation from Methyl Thioethers: Scope, Mechanism, and Relevance for Natural Systems, Chemistry – A European Journal, 23, 10465-10472, 10.1002/chem.201701986, 2017.

Bizic-Ionescu, M., Klintzch, T., Ionescu, D., Hindiyeh, M. Y., Guenthel, M., Muro Pastor, A. M., Keppler, F., and Grossart, H.-P.: Widespread formation of methane by Cyanobacteria in aquatic and terrestrial environments, bioRxiv, 10.1101/398958, 2018.

Bižić-Ionescu, M., Ionescu, D., Günthel, M., Tang, K. W., and Grossart, H.-P.: Oxic Methane Cycling: New Evidence for Methane Formation in Oxic Lake Water, in Biogenesis of Hydrocarbons, Stams A.J.M and Souna D.Z., Springer International Publishing, Basel, 1-22, 2018.

Bogard, M. J., del Giorgio, P. A., Boutet, L., Chaves, M. C. G., Prairie, Y. T., Merante, A., and Derry, A. M.: Oxic water column methanogenesis as a major component of aquatic CH_4 fluxes, 5, 5350, 10.1038/ncomms6350https://www.nature.com/articles/ncomms6350#supplementary-information, 2014.

Borges, A. V., Speeckaert, G., Champenois, W., Scranton, M. I., and Gypens, N.: Productivity and temperature as drivers of seasonal and spatial variations of dissolved methane in the Southern Bight of the North Sea, Ecosystems, 21, 583-599, 2018.

https://doi.org/10.5194/bg-2019-245
Preprint. Discussion started: 1 July 2019

Boros, M., and Keppler, F.: Production and Signaling of Methane, in Gasotransmitters, Wang R., Royal Society of Chemistry, London, 192-234, 2018

Brown, C. W., and Yoder, J. A.: Coccolithophorid blooms in the global ocean, Journal of Geophysical Research: Oceans, 99, 7467-7482, 1994.

Bruhn, D., Møller, I. M., Mikkelsen, T. N., and Ambus, P.: Terrestrial plant methane production and emission, Physiologia Plantarum, 144, 201-209, 2012.

Cho, B.-H., and Komor, E.: The amino acid transport systems of the autotrophically grown green alga Chlorella, Biochimica et Biophysica Acta (BBA)-Biomembranes, 821, 384-392, 1985.

Comba, P., Faltermeier, D., Gieger, S., Keppler, F., Schöler, H. F., and Schroll, M.: Iron catalyzed demethylation of acetic acid, Journal of Coordination Chemistry, 1-14, 10.1080/00958972.2018.1490414, 2018.

Damm, E., Kiene, R. P., Schwarz, J., Falck, E., and Dieckmann, G.: Methane cycling in Arctic shelf water and its relationship with phytoplankton biomass and DMSP, Marine Chemistry, 109, 45-59, 10.1016/j.marchem.2007.12.003, 2008.

Damm, E., Helmke, E., Thoms, S., Schauer, U., Nöthig, E., Bakker, K., and Kiene, R. P.: Methane production in aerobic oligotrophic surface water in the central Arctic Ocean, Biogeosciences, 7, 1099-1108, 10.5194/bg-7-1099-2010, 2010.

Damm, E., Rudels, B., Schauer, U., Mau, S., and Dieckmann, G.: Methane excess in Arctic surface water- triggered by sea ice formation and melting, 5, 16179, 10.1038/srep16179, 2015.

de Angelis, M. A., and Lee, C.: Methane production during zooplankton grazing on marine phytoplankton, Limnology and Oceanography, 39, 1298-1308, 1994.

Denman, K. L., Brasseur, G., Chidthaisong, A., Ciais, P., Cox, P. M., Dickinson, R. E., Hauglustaine, D., Heinze, C., Holland, E., Jacob, D., Lohmann, U., Ramachandran, S., Dias, P. L. d. S., Wofsy, S. C., and Zhang, X.: Couplings between changes in the climate system and biogeochemistry., in: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, edited by: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K. B., Tignor, M., and Miller, H. L., Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 2007.

Donis, D., Flury, S., Stöckli, A., Spangenberg, J. E., Vachon, D., and McGinnis, D. F.: Full-scale evaluation of methane production under oxic conditions in a mesotrophic lake, Nature Communications, 8, 1661, 10.1038/s41467-017-01648-4, 2017.

https://doi.org/10.5194/bg-2019-245
Preprint. Discussion started: 1 July 2019

Field, C. B., Behrenfeld, M. J., Randerson, J. T., and Falkowski, P.: Primary production of the biosphere: Integrating terrestrial and oceanic components, Science, 281, 237-240, 1998.

Florez-Leiva, L., Damm, E., and Farías, L.: Methane production induced by dimethylsulfide in surface water of an upwelling ecosystem, Progress in oceanography, 112, 38-48, 2013.

Forster, G., Upstill-Goddard, R. C., Gist, N., Robinson, C., Uher, G., and Woodward, E. M. S.: Nitrous oxide and methane in the Atlantic Ocean between 50 N and 52 S: latitudinal distribution and sea-to-air flux, Deep Sea Research Part II: Topical Studies in Oceanography, 56, 964-976, 2009.

Gafar, N. A., Eyre, B. D., and Schulz, K. G.: A conceptual model for projecting coccolithophorid growth, calcification and photosynthetic carbon fixation rates in response to global ocean change, Frontiers in Marine Science, 4, 433, 2018.

Ghyczy, M., Torday, C., Kaszaki, J., Szabo, A., Czobel, M., and Boros, M.: Hypoxia-induced generation of methane in mitochondria and eukaryotic cells - An alternative approach to methanogenesis, Cellular Physiology and Biochemistry, 21, 251-258, 2008.

Guillard, R. R., and Ryther, J. H.: Studies of marine planktonic diatoms: I. Cyclotella nana Hustedt, and Detonula confervacea (Cleve) Gran, Canadian journal of microbiology, 8, 229-239, 1962.

Herscu-Kluska, R., Masarwa, A., Saphier, M., Cohen, H., and Meyerstein, D.: Mechanism of the reaction of radicals with peroxides and dimethyl sulfoxide in aqueous solution, Chemistry–A European Journal, 14, 5880-5889, 2008.

Hohenberger, J., Ray, K., and Meyer, K.: The biology and chemistry of high-valent iron–oxo and iron–nitrido complexes, Nature communications, 3, 720, 2012.

Holligan, P. M., Fernández, E., Aiken, J., Balch, W. M., Boyd, P., Burkill, P. H., Finch, M., Groom, S. B., Malin, G., and Muller, K.: A biogeochemical study of the coccolithophore, Emiliania huxleyi, in the North Atlantic, Global Biogeochemical Cycles, 7, 879-900, 1993.

Karl, D. M., and Tilbrook, B. D.: Production and transport of methane in oceanic particulate organic matter, Nature, 368, 732-734, 1994.

Karl, D. M., Beversdorf, L., Bjorkman, K. M., Church, M. J., Martinez, A., and DeLong, E. F.: Aerobic production of methane in the sea, Nature Geoscience, 1, 473-478, 10.1038/ngeo234, 2008.

https://doi.org/10.5194/bg-2019-245
Preprint. Discussion started: 1 July 2019

505 Keeling, C. D.: The concentration and isotopic abundances of atmospheric carbon dioxide in rural areas, Geochimica et Cosmochimica Acta, 13, 322-334, http://dx.doi.org/10.1016/0016-7037(58)90033-4, 1958.

Keller, M. D.: Dimethyl sulfide production and marine phytoplankton: the importance of species composition and cell size, Biological oceanography, 6, 375-382, 1989.

Keppler, F., Hamilton, J. T. G., Brass, M., and Rockmann, T.: Methane emissions from terrestrial plants under aerobic
510 conditions, Nature, 439, 187-191, 2006.

Keppler, F., Boros, M., Frankenberg, C., Lelieveld, J., McLeod, A., Pirttila, A. M., Rockmann, T., and Schnitzler, J. P.: Methane formation in aerobic environments, Environmental Chemistry, 6, 459-465, 10.1071/en09137, 2009.

Keppler, F., Schiller, A., Ehehalt, R., Greule, M., Hartmann, J., and Polag, D.: Stable isotope and high precision concentration measurements confirm that all humans produce and exhale methane, Journal of Breath Research, 10, 016003,
515 2016.

Kirschke, S., Bousquet, P., Ciais, P., Saunois, M., Canadell, J. G., Dlugokencky, E. J., Bergamaschi, P., Bergmann, D., Blake, D. R., Bruhwiler, L., Cameron-Smith, P., Castaldi, S., Chevallier, F., Feng, L., Fraser, A., Heimann, M., Hodson, E. L., Houweling, S., Josse, B., Fraser, P. J., Krummel, P. B., Lamarque, J. F., Langenfelds, R. L., Le Quere, C., Naik, V., O'Doherty, S., Palmer, P. I., Pison, I., Plummer, D., Poulter, B., Prinn, R. G., Rigby, M., Ringeval, B., Santini, M., Schmidt,
520 M., Shindell, D. T., Simpson, I. J., Spahni, R., Steele, L. P., Strode, S. A., Sudo, K., Szopa, S., van der Werf, G. R., Voulgarakis, A., van Weele, M., Weiss, R. F., Williams, J. E., and Zeng, G.: Three decades of global methane sources and sinks, Nature Geoscience, 6, 813-823, 10.1038/ngeo1955, 2013.

Langer, G., Nehrke, G., Probert, I., Ly, J., and Ziveri, P.: Strain-specific responses of Emiliania huxleyi to changing seawater carbonate chemistry, Biogeosciences, 6, 2637-2646, 2009.

525 Langer, G., Oetjen, K., and Brenneis, T.: Calcification of Calcidiscus leptoporus under nitrogen and phosphorus limitation, Journal of Experimental Marine Biology and Ecology, 413, 131-137, 2012.

Langer, G., Oetjen, K., and Brenneis, T.: Coccolithophores do not increase particulate carbon production under nutrient limitation: A case study using Emiliania huxleyi (PML B92/11), Journal of experimental marine biology and ecology, 443, 155-161, 2013.

530 Laukenmann, S., Polag, D., Heuwinkel, H., Greule, M., Gronauer, A., Lelieveld, J., and Keppler, F.: Identification of methanogenic pathways in anaerobic digesters using stable carbon isotopes, Engineering in Life Sciences, 10, 509-514, 10.1002/elsc.201000074, 2010.

Lelieveld, J., Crutzen, P. J., and Dentener, F. J.: Changing concentration, lifetime and climate forcing of atmospheric methane, Tellus B, 50, 128-150, 1998.

535 Lenhart, K., Bunge, M., Rathering, S., Neu, T., Schüttman, I., Greule, M., Kammann, C., Schnell, S., Müller, C., Zorn, H., and Keppler, F.: Evidence for methane production by saprotrophic fungi, Nature Communications, 3, 10.1038/ncomms2049, 2012.

Lenhart, K., Althoff, F., Greule, M., and Keppler, F.: Technical Note: Methionine, a precursor of methane in living plants, Biogeosciences, 12, 1907-1914, 10.5194/bg-12-1907-2015, 2015a.

540 Lenhart, K., Weber, B., Elbert, W., Steinkamp, J., Clough, T., Crutzen, P., Pöschl, U., and Keppler, F.: Nitrous oxide and methane emissions from cryptogamic covers, Global Change Biology, 21, 3889-3900, 10.1111/gcb.12995, 2015b.

Lenhart, K., Klintzsch, T., Langer, G., Nehrke, G., Bunge, M., Schnell, S., and Keppler, F.: Evidence for methane production by the marine algae Emiliania huxleyi, Biogeosciences, 13, 3163-3174, 10.5194/bg-13-3163-2016, 2016.

Liss, P., Malin, G., Turner, S., and Holligan, P.: Dimethyl sulphide and Phaeocystis: a review, Journal of Marine Systems, 5,
545 41-53, 1994.

Martel, A. B., and Qaderi, M. M.: Light quality and quantity regulate aerobic methane emissions from plants, Physiologia plantarum, 159, 313-328, 2017.

Matrai, P. A., and Keller, M. D.: Dimethylsulfide in a large-scale coccolithophore bloom in the Gulf of Maine, Continental Shelf Research, 13, 831-843, 1993.

550 Menden-Deuer, S., and Lessard, E. J.: Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton, Limnology and oceanography, 45, 569-579, 2000.

Metcalf, W. W., Griffin, B. M., Cicchillo, R. M., Gao, J. T., Janga, S. C., Cooke, H. A., Circello, B. T., Evans, B. S., Martens-Habbena, W., Stahl, D. A., and van der Donk, W. A.: Synthesis of Methylphosphonic Acid by Marine Microbes: A Source for Methane in the Aerobic Ocean, Science, 337, 1104-1107, 10.1126/science.1219875, 2012.

555 Olenina, I., Hajdu, S., Edler, L., Andersson, A., Wasmund, N., Busch, S., G¨obel, J., Gromisz, S., Huseby, S., Huttunen, M., Jaanus, A., Kokkonen, P., Ledaine, I., and Niemkiewicz, E.: Biovolumes and size-classes of phytoplankton in the Baltic Sea, HELCOM Baltic Sea Environ. Proc., 106, p. 144, 2006.

Oremland, R. S.: Methanogenic activity in plankton samples and fish intestines – mechanism for insitu methanogenesis in oceanic surface waters, Limnology and Oceanography, 24, 1136-1141, 1979.

560 Pataki, D. E., Ehleringer, J. R., Flanagan, L. B., Yakir, D., Bowling, D. R., Still, C. J., Buchmann, N., Kaplan, J. O., and Berry, J. A.: The application and interpretation of Keeling plots in terrestrial carbon cycle research, Global Biogeochemical Cycles, 17, n/a-n/a, 10.1029/2001GB001850, 2003.

Paul, D., Skrzypek, G., and Fórizs, I.: Normalization of measured stable isotopic compositions to isotope reference scales – a review, Rapid Communications in Mass Spectrometry, 21, 3006-3014, 10.1002/rcm.3185, 2007.

565 Reeburgh, W. S.: Oceanic methane biogeochemistry, Chemical Reviews, 107, 486-513, 10.1021/cr050362v, 2007.

Repeta, D. J., Ferrón, S., Sosa, O. A., Johnson, C. G., Repeta, L. D., Acker, M., DeLong, E. F., and Karl, D. M.: Marine methane paradox explained by bacterial degradation of dissolved organic matter, Nature Geoscience, 9, 884, 2016.

Saunois, M., Bousquet, P., Poulter, B., Peregon, A., Ciais, P., Canadell, J. G., Dlugokencky, E. J., Etiope, G., Bastviken, D., and Houweling, S.: The global methane budget 2000–2012, Earth System Science Data, 8, 697-751, 2016.

570 Schmale, O., Wäge, J., Mohrholz, V., Wasmund, N., Gräwe, U., Rehder, G., Labrenz, M., and Loick-Wilde, N.: The contribution of zooplankton to methane supersaturation in the oxygenated upper waters of the central Baltic Sea, Limnology and Oceanography, 63, 412-430, 2018.

Schoemann, V., Becquevort, S., Stefels, J., Rousseau, V., and Lancelot, C.: Phaeocystis blooms in the global ocean and their controlling mechanisms: a review, Journal of Sea Research, 53, 43-66, 2005.

575 Scranton, M. I., and Brewer, P. G.: Occurrence of methane in the near-surface waters of the western subtropical North-Atlantic, Deep Sea Research, 24, 127-138, http://dx.doi.org/10.1016/0146-6291(77)90548-3, 1977.

Scranton, M. I., and Farrington, J. W.: Methane production in the waters off Walvis Bay, Journal of Geophysical Research, 82, 4947-4953, 10.1029/JC082i031p04947, 1977.

Spiese, C. E., Le, T., Zimmer, R. L., and Kieber, D. J.: Dimethylsulfide membrane permeability, cellular concentrations and
580 implications for physiological functions in marine algae, Journal of Plankton Research, 38, 41-54, 2015.

Stawiarski, B., Otto, S., Thiel, V., Gräwe, U., Loick-Wilde, N., Wittenborn, A. K., Schloemer, S., Wäge, J., Rehder, G., and Labrenz, M.: Controls on zooplankton methane production in the central Baltic Sea, Biogeosciences, 16, 1-16, 2019.

Stefels, J., Steinke, M., Turner, S., Malin, G., and Belviso, S.: Environmental constraints on the production and removal of the climatically active gas dimethylsulphide (DMS) and implications for ecosystem modelling, Biogeochemistry, 83, 245-275, 10.1007/s10533-007-9091-5, 2007.

Sunda, W., Kieber, D. J., Kiene, R. P., and Huntsman, S.: An antioxidant function for DMSP and DMS in marine algae, Nature, 418, 317-320, 10.1038/nature00851, 2002.

Tang, K. W., McGinnis, D. F., Frindte, K., Brüchert, V., and Grossart, H.-P.: Paradox reconsidered: Methane oversaturation in well-oxygenated lake waters, Limnol. Oceanogr, 59, 275-284, 2014.

Thomsen, H. A. Buck, K.R. and Chavez F.P.: Haptophytes as components of marine phytoplankton, in: The Haptophyte Algae, Green J.C. and Leadbeater B.S.C., Clarendon Press, Oxford, 187-208, 1994

Weller, D. I., Law, C. S., Marriner, A., Nodder, S. D., Chang, F. H., Stephens, J. A., Wilhelm, S. W., Boyd, P. W., and Sutton, P. J. H.: Temporal variation of dissolved methane in a subtropical mesoscale eddy during a phytoplankton bloom in the southwest Pacific Ocean, Progress in Oceanography, 116, 193-206, http://dx.doi.org/10.1016/j.pocean.2013.07.008, 2013.

Zhang, Y., and Xie, H.: Photomineralization and photomethanification of dissolved organic matter in Saguenay River surface water, Biogeosciences, 12, 6823-6836, 10.5194/bg-12-6823-2015, 2015.

Zindler, C., Bracher, A., Marandino, C. A., Taylor, B., Torrecilla, E., Kock, A., and Bange, H. W.: Sulphur compounds, methane, and phytoplankton: interactions along a north-south transit in the western Pacific Ocean, Biogeosciences, 10, 3297-3311, 10.5194/bg-10-3297-2013, 2013.