Replies to Comments on “Controls on zooplankton methane production in the central Baltic Sea” by Stawiarski et al., manuscript bg-2018-345

We sincerely thank both reviewers for their insightful comments on our manuscript, which have greatly helped to clarify our findings. The main changes made to our manuscript include:

- a shorter introduction which focuses on the main information which is essential for understanding the topic.

- discussing organic sulphur compounds generally as potential CH_4 source, e.g. inclusion of DMSO besides DMS and DMSP as methane precursors.

- inclusion of the locations of the stations in figure 6 for considering upwelling as being influential on the plankton community composition

- inclusion of an equation for calculating methane production rates

- discussion about the minimization of stress factors for the physiological response of the animals

Please find below the *original comments* (in italics) along with our replies (in standard).

On behalf of our co-authors with best regards from Rostock,
Oliver Schmale and Beate Stawiarski

Referee #1

General comments:

[...] The results presented shed new light on the still enigmatic accumulation of methane in oxic marine environments. The manuscript is well written and concise and the majority of the conclusions are justified by the presented data. However, I have a few major concerns (see pts 1), 9)-11) below)and, therefore, I can recommend publication only after major revisions.

We would like to thank the reviewer for acknowledging the value of our work and hope that the changes, which were applied will help to clarify the concerns.

Specific comments:
1) Introduction: The introduction looks more like a review. A lot of details are given; however, they do not help at all to place the new data/results in a broader context. I suggest to shorten the introduction and to focus it on the main points which are relevant for the discussion of the results.

We agree with the reviewer's suggestion and changed to introduction accordingly (see revised manuscript).

2) Introduction: The photochemical source of CH4 is not mentioned. This source might be important in the Baltic Sea in view of the fact that the surface layers of the Baltic Sea are heavily influenced by rivers and rich in CDOM, see [Zhang and Xie, 2015].

We agree with the reviewer's comment and added the photochemical source as follows to the list of methane production pathways:

“(v) photochemical production of methane from colored dissolved organic matter (chromophoric dissolved organic matter, Zhang and Xie, 2015)”

The importance of CDOM in the Baltic Sea as a methane precursor might be interesting, but is not subject of this study, that focuses on the zooplankton associated methane production in the central Baltic Sea, where an immediate influence by river plumes cannot be expected. We agree with the reviewer that future investigations in the Baltic Sea are needed to study the importance of CDOM as a possible methane precursor in river plumes.

3) Introduction: DMSO can be a precursor for CH4 as well, see e.g. [Zindler et al., 2013]. This source should be mentioned.

We thank the reviewer for making us aware of this additional precursor.

We added DMSO to the Introduction as follows:
"(iv) CH_4 production through microbial degradation of dimethylsulfide (DMS), dimethylsulfoniopropionate (DMSP) and dimethylsulfoxide (DMSO) (Damm et al., 2010, Zindler et al. 2013)."

We also added the following statement to the discussion:
"Dinophyceae, in particular the mixotrophic Dinophysis norvegica, were more abundant at stations with a distinct subthermocline methane enrichment. Dinophyceae produce relatively high amounts of DMSP and DMSO compared to the other phytoplankton species observed within our study (Keller et al. 1989, Hatton and Wilson 2007, Caruana & Malin 2014). Also, positive correlations have previously been observed between DMSP and CH_4 and DMSO and CH_4 in the surface ocean (Zindler et al, 2013)."

4) Page 3, lines 5/6: The statement that ocean and lakes contribute about 20% to the global natural CH4 emissions is strongly misleading. The Baltic Sea is not a lake, thus, its emissions contribute to the oceanic/coastal CH4 emissions which, in turn, contribute only 1% or less to the global CH4 sources. Oceanic emissions are, therefore, a minor source of atmospheric CH4. In the latest IPCC Report (which is also cited in the manuscript) oceanic emissions are not mentioned as a separate source.

We agree with the reviewer and deleted the statement from the manuscript.

5) P3L14-20: The role of methylphosphonate (MPn) as source of CH4: There are contradicting results: Valle and Karl [del Valle and Karl, 2014] showed that CH4 is not produced from dissolved MPn but rather from particulate/particle-bound MPn (pMPn); which seems to be in contrast to the results by Repeta et al., 2016.

We shortened the introduction to include information, which is essential for the discussion of the presented data. In this way, we reformulated our statement to be more general and included further references "(ii) bacterial break-down of methylphosphonate (MPn) under phosphate-stressed conditions (Repeta et al., 2016; Karl et al., 2008; Wang et al., 2017; Teikari et al., 2018)"

6) P9L30: '… since it represents.' I think a part of the sentence is missing.

This is correct. We deleted the sentence from the Introduction of the manuscript.

7) P12L7: Please remove 'sea/air exchange' from the subtitle. The air/sea exchange of CH4 (i.e. CH4 fluxes) is not presented or discussed at all.

We changed the title to "3.1 Subthermocline methane distribution"

8) P12L9/10: please present an equation (incl. a ref for the solubility of CH4) for the calculation of the CH4 saturations. (the equation should be placed to the Method section).

We have added the equation in section "2.1. Hydrographical and chemical characteristics of the water column".

Surface water methane saturation is calculated following Eq. (1), where SV is the saturation value, Cw is the measured concentration of methane in seawater and Cequi the concentration in equilibrium with the atmosphere using the solubility coefficient given by Wiesenburg et al. (1979).

$$SV[\%] = \frac{C_w}{C_{equi}} * 100 \qquad (1)$$

9) P15L5-7: In view of the large standard deviations, the given averages do not seem to be significantly different. Therefore, the conclusion of a composition-depending CH4 production is not justified. Please provide the results of a statistical test which shows that the two averages are significant indeed.

The reviewer noticed correctly that the averages are not statistically significant. We mentioned that the rates were "higher", but did not present any statistics. For clarity we changed the paragraph as follows:

"The incubations with a high proportion of T. longicornis had higher production rates than the Acartia spp. dominated setups (125 ±49 vs. 84 ±19 fmol CH4 copepod-1 d-1). This indicates that methane production may depended on the composition of the zooplankton community (Fig. 6a). However, the differences were not significant (Kruskall Wallis Test (p=0.150, df=1), which may be a consequence of the limited number of incubations."

10) P16L4/5: '… may have reflected a response of the animals to stress of being removed from their natural environment'. This statement questions all of the presented results. When the zooplankton is that much stressed, how did the authors make sure to get reliable/representative results?

We are aware that experiments with natural communities may cause stress to the animals. This general problem needs to be addressed by any researcher who conducts similar physiological experiments. Within our experiments we tried to avoid capture and food stress, which were identified to be influential. For clarity we included the following paragraph:

"To lower the capture stress we selected a rather gentle method for sampling and we avoided food shortage, which was shown to be more influential on the decrease in physiological rates (Ikeda and Skjoldal, 1980). Also, we used a food source which was previously shown to be of good quality (e.g. Knuckey et al. 2005 , Koski and Breteler 2003)."

However, we are aware that the rates obtained in our artificial experiments can never be transferred directly into the natural environment but can serve as an essential contribution to study the factors controlling the shallow water methane production.

11) P17, discussion. I agree with the line of arguments for DMSP as a potential CH4 precursor. However, there might have been other particle-bound potential CH4 precursors around, e.g. DMSO and MPn. As said above, del Valle and Karl (2014) showed that pMPn is resulting in CH4 production. So, I wonder whether the discussion is only considering DMSP.

We agree with the reviewer's concerns and adjusted the title of the discussion to include different organic sulfur compounds and included DMSO as a possible methane precursor in the discussion section:

"Organic sulfur compounds as possible substrates for methane production in oxic waters"

"They (Dinophyceae) produce relatively high amounts of DMSP and DMSO compared to the other phytoplankton species observed within our study (Keller et al. 1989, Hatton and Wilson 2007, Caruana & Malin 2014). Also, positive correlations have previously been observed between DMSP and CH4 and DMSO and CH4 in the surface ocean (Zindler et al, 2013)."

Teikari at al. (2018) recently found that N. spumigena could produce CH_4 when grown on MPn as phosphorus source. Since cyanobacteria are accumulated in the surface water it might be possible that the process described by Teikari et al. (2018) has an impact on methane productionin the surface waters (above the thermocline). This agrees with the methane budget calculation published by Schmale et al. (2018), who point towards an additional shallow methane source, which is required to maintain the measured methane flux into the atmosphere. However, it is difficult to explain how cyanobacteria related CH_4 production can substantially contribute to the observed CH_4 enrichment below the thermocline, where the biomass of cyanobacteria is comparably low. Furthermore, there are currently no data available about the quantitative distribution of MPn in surface waters in the Baltic Sea. Based on this knowledge gap, we suggest that further studies should investigate the importance of MPn as a possible methane precursor in Baltic Sea surface waters.

12) P17L19: The authors ‘believe’? We are scientists, that means we discuss results on the basis of arguments. Please rephrase.

We agree with the reviewer's comment and changed the sentence accordingly:

"We therefore suggest that anaerobic methanogenesis by archaea thriving within fecal pellets played only a minor role."

13) P24L23-27: This part of the text should be moved to the Conclusion section.

We moved the suggested section to the conclusions. In addition, we shortened the conclusions section to be more concise.