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 #2

General comments:

Stawiarski et al. present measurements and experimental data, which they obtained to identify the origin of elevated methane concentrations in the oxic subsurface water of the Baltic Sea. They tested a hypothesis forwarded by Schmale et al. (2018), if spatial heterogeneity of subsurface methane concentrations result from differences in the copepod community and the associated food web. Therefore, they sampled zooplankton and phytoplankton. They incubated zooplankton communities of different surface and subsurface waters, fed them specific phytoplankton, and derive the methane production rates. Their experiments show that fed zooplankton generate methane and additional methane might be generated by microbial turnover of DMSP. **Overall, it is a well-designed study and manuscript. I found it very interesting to read and have only a few comments.**

We would like to thank the reviewer for acknowledging the value of our work and hope that the changes, which we applied will address the suggested comments to the reviewer's satisfaction.

Specific comments:

1) The spatial heterogeneity of the subsurface methane peak is partly discussed as the result of upwelling (page 14). In figure 4, the authors show the development of the surface water temperature indicating upwelling along the Swedish coast and

southeast of Gotland. They relate the high methane concentrations in the surface water at station TF0284 to the upwelling, but do not discuss the other stations. If I consider the positions of the stations, upwelling might have influenced the waters at station TF0283, too. Therefore, I suggest 1) to add the locations of the other stations to figure 4 (longitudes and latitudes are missing on the maps, too) to identify where upwelling might have affected the subsurface methane concentration. Therefore, the spatial heterogeneity might be mainly due to upwelling and not a consequence of different plankton communities and 2) consider the increased mixing to modify the plankton community.

We agree with the reviewer's concerns and applied the following changes according to the suggestions:

a) we added the other locations of the other stations to figure 4 and added longitudes and latitudes. These changes helped us to identify that no station was affected by upwelling during our time of sampling. Station TF0283 was indeed at the edge of the upwelling front, but from its temperature depth profile we suggest that the sampled water body must not have been affected, yet. We included the following statement:

“The other stations were not affected by upwelling events during the time of sampling. Even though our oceanographic model output indicated that the water mass at station TF0283 (sampled on the 11th of August) was located at the upwelling front (Fig. 4), our field measurements showed that the station was not affected by the event, as there is no drop in the surface water temperature visible (Fig. 3).”

b) We added the following statement to the introduction:
“Upwelling events can offset water column stratification through a replacement of warm, mostly nutrient-depleted surface water by cooler and usually nutrient-enriched subthermocline waters (Gidhagen, 1987; Lehmann and Myrberg, 2008; Reissmann et al., 2009). Such events may also cause a rapid decline in phytoplankton biomass in the surface water and affect the plankton composition (Vahtera et al. 2005, Nausch et al. 2009, Wasmund et al. 2012).”

c) We also added this statement to the results and discussion:
"However, the phytoplankton biomass was lower at station TF0284, which was recently influenced by an upwelling event. Hence, it needs to be considered that also the phytoplankton composition at this station could have been altered by the event."

2) The calculation of the specific activity of the phytoplankton (page 9) includes three terms that are constants, thus, can be ignored and only the first term, i.e. disintegration filter, which is the activity of the phytoplankton, should be presented in table 1. The three constants are the constant for converting DPM to MBq, the added activity, and the specific activity of the tracer.

We agree with the reviewer's comment. However, two out of the three mentioned constants must or may be modified in future experiments according to culture volume and activity of the

tracer. Hence, for better and easier reproducibility we would like to keep the equation in its current form. The present equation (Eq. 2) is also identical with the equation listed in our reference paper published by de Angelis and Lee (1994), who performed similar incubations to measure zooplankton methane production rates.

3) Besides, how was the constant for converting DMP to MBq derived? It appears as if a quench correction is included, but usually, the quench of each sample is slightly different and is corrected by applying a quench curve. How was the quench determined?

We used the method described by Jakobs et al. (2013) along with the same equipment and chemicals. Hence we were also able to use the quench curve obtained by those authors.

4) Furthermore, how did you derive the specific activity of the tracer (SAtracer)? Did you measured it or did you use the value provided by the manufacturer? In my experience, the latter is erroneous and should be validated by own measurements.

We and other working groups in our institute made a different experience regarding the quality of radiolabels from this company. The quality of the radiolabel (NaHCO3) was confirmed in different studies before and was also checked in the present study. We used the specific activity provided by the manufacturer, which was obtained individually for our solution.

5) Anyway, the activity of the phytoplankton is needed to calculate the methane production rate, but an equation of the rate is not included in the manuscript. Therefore, please review equation 1 and include an equation of the methane production rate.

We thank the reviewer for this hint and added an appropriate equation (Eq. 3) to the manuscript.

6) Are there any more accurate numbers for the percentages of Cryptophyceae and N. spumigena (page 10)? It is a very broad statement to distinguish between minor and dominant percentage to the total phytoplankton.

To the best of our knowledge no paper that presents any more numbers for the abundance of Cryptophyceae in the central Baltic Sea is available. Based on the IOW monitoring program we calculated the percentage of Cryptophyceae of the total phytoplankton biomass and added the following text:

"Rhodomonas sp. may be considered a model representative of the Cryptophyceae, which account for 5.5 % of the total phytoplankton biomass in the Baltic Sea in summer 2016 (IOW monitoring database: https://www.io-warnemuende.de/datenportal.html)."

For the numbers of *N. spumigena* we added the following sentence:

“Here we selected *N. spumigena* as a food source, because this species was the dominant phytoplankton in the surface waters during our field campaign and accounted for 23% of the phytoplankton biomass.”

7) Please include if the incubations were done in the dark or not (page 10).

The incubations were done in the dark. We included the following statement:

“All bottles were kept in the dark at in situ temperature for 1 to 3 days in temperature controlled incubators.”

8) The difference between the measured methane production rates and the ones reported by de Angelis and Lee (1994) (page 16) might not be due to stressed copepods, but might result from filtration of the seawater (page 25). De Angelis and Lee (1994) most likely set up a similar experiment; therefore, their copepods were similarly stressed. Nevertheless, did they use filtered or unfiltered seawater? Could this explain the difference?

De Angelis and Lee (1994) used GF/C filtered seawater for their experiments. However, their pore size was slightly bigger (1.2µm) than within our experiments (GF/F, 0.7µm). We included the following statement:

“Another factor which may have led to lower methane production rates than measured by de Angelis and Lee (1994) is the quality of the filtered sea water used in the incubations. In our experiments we used filters with a pore size of 0.7 µm while de Angelis and Lee (1994) used filters with a pore size of 1.2 µm to prepare the incubation water. Our intention was to exclusively investigate the methane production by zooplankton while minimizing the influence of particulate material (e.g. fecal pellets) in the seawater. However, we are aware that the smaller pore size used in our studies may reduced the number of bacteria in the incubation water, which may have been important for the methane production outside the body of the copepods.”

9) Typos: P2L6: space missing between _ and 6.1 P6L26: space missing between 1.5 and L

We included a space.

P6L29: Did Schmale et al. (2018) study the migration of copepod T. longicornis as suggested by citing the paper?

The reviewer noticed correctly that the citation was incorrect. We deleted it.

Table 1: It appears as if the duration of experiment 1 with surface zooplankton community is missing.

We moved the duration of the experiment to the center of the cell to be in line with "Exp." and "SAphy"

P10L3: space missing between Fig. and 8

We refurmulated this section.

P12L21: subthermocline, the r is missing

We included an "r" in "subthermocline".