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Interactive comment on "Methane production by three widespread marine phytoplankton species: release rates, precursor compounds, and relevance for the environment" *by* Thomas Klintzsch et al.

Anonymous Referee #2

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The present paper presents an interesting study about methane production under oxic conditions in marine environments. This so called "methane paradox" is a very important research field to understand methane emissions from oceans (and lakes) and has recently received strong interest by a number of investigators from different scientific disciplines. The author presents data from incubation experiments conducted with three different algal species. Methane production rates were determined with different methanogenic substrates (13C-labled) using a stable isotope approach. A similar kind of studies was previously conducted for Emiliania huxleyi by Lenhart et al. (2016)

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and the isotope approach was successfully used in diverse investigations by Frank Keppler before to examine terrestrial methane production. The novel outcome in the present study is (1) that also other widespread haptophytes have the potential to produce methane under anoxic conditions; and (2) methylated sulphur compounds (e.g. DMS), that are known to be enriched in the investigated algae species, present potential substrates. In addition, the authors present an attempt to transfer their results to an algal bloom in the Pacific Ocean to discuss the potential relevance of algal methane production.

The experiments are well thought out and the results present an additional piece in the complex puzzle. There are lots of little corrections needed and from my point of view some sentences need another structure to make the content more accessible for readers that are not familiar with the topic in detail (especially in the method section, e.g. PP, exponential growth rate). I will give a few examples below.

Some minor and major points need to be addressed and I therefor recommend a publication after major revision.

Major issues:

Line 95ff. The experimental design is very complex. A flowchart for the method section would be helpful for the reader.

Line 98ff. How clean are the algal culture samples (purity)? Small differences in the degrees of contamination with archaea/bacteria (nitrogen limited bacteria, Line 69, Damm) between the cultures may have an impact on CH4 production rate. Does the web link give information about the purity of the culture?

Line 133. What is the difference in concentration of NaHCO3 between natural and inoculated water sample? Why did the authors added this amount of tracer? Should be mentioned.

Line 137. Can you explain if aggregates or sediment was visible in the incubation?

Line 141. Here, you should explain in more detail why an exponential growth rate is important to best compare CH4 formation between the experiments. From this sentence one could assume that Langer performed already methane production rate experiments with algae that indicated that exponential growth rates are important. From my point of view the activity of the cell is important for the turnover of these substrates and not their reproduction.

It should be mentioned in the method or result section that microbial methane turnover takes place in the incubations and the production rates presented are minimum rates > because methane oxidation is not considered in the calculations (e.g. see methods in de Angelis and Lee).

Line 172. Why was exactly this amount of substrate (DMS. . .) injected and is this comparable with natural environments (concentrations). Substrate concentrations definitely affect the turnover and the addition of tracers/substrates should not impact the sample too drastically. Why did the authors did not applied MET (and DMSP) as a precursor that was tested before successfully by Lenhart et al.?

Line 327ff. Is it possible that a natural microbial community is needed for the turnover of these substances to methane? If the incubations are without contaminations (sterile filtered seawater, pure culture), the production rates might be low because of the missing community. The algae may only provide the precursors. Might be a point that could be discussed here.

Line 335. I have a different impression. Figure 4a: At day 2 the d13C values are very close to each other. In Figure 4b all values from beginning to the end of the incubation time are very similar. Only Figure 4c shows a clear difference between culture and control over the course of the experiment. Add in the figure caption that also controls are plotted, not only results from cultures.

Line 381. Argumentation is difficult. Only because Lenhart could prove a contamination-free incubations, this result cannot be transferred to all the incubation

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that will be performed by the working group afterwards. See comments/concerns to this topic above. Since the argumentation is difficult to follow, I suggest to discuss this topic less dominant and integrate this part somewhere else (not under a separate title). Also 50% of the text is nearly copied from the introduction (doubling!).

Chapter 4.3 I would recommend to perform an additional calculation to show that algal CH4 production is an important mechanism that can explain air/sea methane fluxes and methane enrichments. For example Schmale et al. (2018) gives detailed data about phytoplankton biomass (e.g. Prymnesiales) and production rates needed to maintain air/sea fluxes and subthermocline methane enrichments. There are probably also other papers available that could be used for such calculation.

Minor issues:

Title: I recommend writing “potential relevance for the environment”. A direct transfer of laboratory studies/results into field observations is difficult.

Line 24. Please also give the productions rates per cell in the abstract. Temperature is not needed to be mentioned in the abstract.

Line 27ff. It should be mentioned here that the conversion of methylated sulphur compounds to methane was only responsible for less than 1% of the observed methane production (line 327ff).

Line 26-29. The word “clearly” is used to often.

Line 30. “Relevance for the environment” is one major issue in the title but is reduced here to a little sentence. This part should be extended.

Line 49. How can “emissions from freshwater” explain the CH4 concentration in ocean surface water? Line 50. Shorten the sentence and delete “that has been often. . .”. “Well-known” means “often reported”

Line 55-58. This paragraph should be moved to line 46. It might be better to start with

this overall review before listing the recent specific studies to oxic methane production in lakes and ocean.

Line 60. May also mention Valle and Karl (2014) who used in situ MPn concentration in a 14C approach and showed that dissolved MPn in surface waters cannot account for methane oversaturation.

Line 98. A bracket is missing (RC. . .). Is it clear for the reader for what the web link is good for?

Line 102. Delete “in” in front of “natural”

Line 110ff. Why did the authors used different volumes (medium and headspace)?

Line 119. What is meant with “main cultures”? Is this the investigated culture in the incubation?

Line 119ff. I would suggest to transfer the cell densities to the result section (3.1).

Line 122. E. huxleyi was sampled daily! What do you mean with overall sampling interval: 9,11,6 days? Why this odd order? And why did you sample the cultures in different intervals? If there is a reason for that it should be explained.

Line 128. Why only three data points for E. huxleyi. From Figure 1c it seems to be plausible to use four.

Line 131. It is always worth to have a repetition to support the previous results.

Line 134. The delta is missing in d13C

Line 135. Suggest to write: “. . .values of the methane precursor. . .”

Line 144. “. . .measured at the end. . .”

Line 145. Suggest to write “For this additional experiment. . .”

Line 146. Suggest putting the cell densities in the result section (see above).

Line 153. “ag” is the abbreviation for what?

Line 158. The program “Image J” is produced by which company?

Line 174. I still think that cell densities should be implemented in the result section (see above).

Line 176. The target/design of the experiment in section 3.2 is still unknown!

Line 189. Analyzed. See line 180

Line 193. Write: ”. . . (based on three. . .)”.

Line 204. Delete: “. . .at a temperature. . .”. Here and in some other parts of the result section you mention details that were mentioned before in the method section.

I would start with a sentence that makes clear that you are talking about the incubation with 13C-labelled hydrogen carbon (2.3).

Line 208. Also here delete the repeated information: “These rates were obtained. . .”. Check the entire result section to avoid redundancy.

Line 211. The cell density should only be mentioned here and not in the method section!

Line 214. Where is the control group plotted?

Figure 2. Black and blue dots are difficult to distinguish. Even if it is “only” the control sample – make the visibility easier. The x-axis should be 1/CH4. Right?

Line 249. See above. Not clear why the exponential growth phase is important and not the cell activity.

Line 251. The equation is already described above (2 and 4). Avoid doubling. See comment above.

Line 254. The sentence should end with (Tab. 1).

Line 256. “community level” sounds odd in this context. May you can find a better description.

Line 266. It starts again with information that was mentioned before in the methods.

Line 271. The sentence should end with “(Fig. 4)”.

Figure 4b. Change the x-axis to 13C2 (add 2).

Figure 4c. Change the x-axis to MSO (not MES, see caption).

Line 279. Add the control sample in the Figure.

Line 302. In the present study only the turnover of 13C-hydrogen carbonate by two algal species was investigate. Lenhart applied the isotope technique for E. huxleyi.

Line 307. (with highest cell numbers) is out of context. Please rephrase.

Line 333. In future investigations I would suggest a dark incubation to exclude methane production by UV or visible light (line 70ff).

Line 352ff. Did Althoff really proved that the “reactivity” is the driving force in her experiments? Or are point 1 (label concentration) and 2 (penetration) also possible explanations for her observations?

Line 360ff. Sentence too complex. Devide in two parts.

Line 363 and 365. Too often “furthermore”. Rephrase.

Line 391. Interesting. But it needs be explained in more detail why the growth rate impacts the methane production. See above.

Line 411. Include/explain why PP is meaningful parameter.

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