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Interactive comment on "Impacts of biogenic polyunsaturated aldehydes on metabolism and community composition of particle-attached bacteria in coastal hypoxia" *by* Zhengchao Wu et al.

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1. However, there appeared to be some problems about the experimental design of this study and the manuscript fails to provide convincing results.

Response: We have carefully addressed the reviewer's comments on our experimental design and the related data and results. Please refer to our detailed responses to each of these specific comments below.

2. More details about the motivation and experiment procedure should be included and

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clarified and the conclusions should be carefully justified.

Response: We thank the reviewer for this suggestion. We have carefully rewritten these parts of the manuscript in the introduction and the method sections to clarify the motivation of our study and provide the details of the experiment setup, operational procedure, and relevant methodology. We have also rewritten the conclusion section as the review suggested. Please refer to the specific comments of this reviewer below for details of our revisions in each section.

3. My major concerns about this manuscript is that the authors consider PAB as bacteria attached to particles with a size >25 μm in the microcosm incubation. A great variety of bacteria would be lost, which would affect the major conclusion of the manuscript. The abundance of bacteria attached to >25 μm particles would be significantly lower than that of free-living bacteria.

Response: We completely agree with the reviewer that a complete PAB community should be acquired using a smaller filtration such as 1 ïĄ■m. Actually, in the high turbid estuarine waters of the PRE, PAB on the particle size of > 25 ïĄ■m could be only about 20 percent of the PAB on the particle size of > 2 ïĄ■m (Ge et al., 2020). Meanwhile, we should emphasize that our primary goal is to explore the mechanism for PUA affecting PAB variation and associated oxygen consumption in high turbidity and low oxygen regions of the PRE. Although the PUA was nanomolar in the bulk water, it can reach a micromolar level on the surface of the particles where they are produced. The hotspot PUA concentration accumulated on the particle surface may increase along with the growth of particle aggregations, varying from 1 ïĄ■mol L-1 to more than 100 ïĄ■mol L-1 (Edwards et al., 2015). Therefore, we chose the larger aggregates with the particle size of > 25 ïĄ■m in the hypoxic waters to perform the PUA-amended experiments, in order to better explore the PUA effects on PAB in the hypoxic waters. We agree that a more systematic study in the future may need to investigate PUA impacts on PAB associated with the particle size of >1 ïĄ■m. Future study may also need to explore the impact of nanomolar PUAs on the free-living bacteria in the background bulk waters.

4. Also, is this particle size proper for measurement of polyunsaturated aldehydes?

Response: It is specific for particle-adsorbed PUAs on particles of > 25 ïĄ■m. A previous study by Edwards et al (2015) uses an even larger size of 50 ïĄ■m for collecting sinking particles for PAB and the associated estimation of hotspot PUAs concentration.

5. In addition, it is not clear why the authors choose 1 or 100 μmol L-1 but not the background value for the incubation.

Response: We should emphasize that the concentration of PUA in the water-column is inhomogeneous due to the presence of particles. Although the PUA was nanomolar in the bulk water, it can reach a micromolar level on the surface of the particles where they are produced. The micromolar level of PUA for incubation was chosen to represent the actual hotspot concentration of PUA (the PUA concentration in the volume of the water parcel taken up by the aggregation particles) not the mean PUA concentration (nanomolar level) in the bulk water.

6. Moreover, bacterial community of the initial inoculates was lacking.

Response: In the revised manuscript, we have provided the initial bacterial community data (T=0) for the experiment in the supplementary material (Figure S2).

7. Personally, the most interesting part of this study is the role of polyunsaturated aldehydes-enhanced bacterial oxygen demand for the seasonal hypoxia. Thus, it is important to know to what extent different concentration of polyunsaturated aldehydes affect bacterial growth and respiration. Although the authors provide discussion on this, more analyses including the selection of background concentration of polyunsaturated aldehydes and testing on pure isolates are needed.

Response: We thank the reviewer for this suggestion. In the revised manuscript, we have provided a discussion on the impact of the background nanomolar level of PUAs on bacteria activity. “The effect of background nanomolar PUAs on free-living bacteria was not explored during our study. Previous studies of the coastal bacterial commu-

nities in the NW Mediterranean Sea suggested that 7.5 nmolL-1 PUAs would have a different effect on the metabolic activity of distinct bacterial groups although bulk bacterial abundance remained unchanged (Balestra et al., 2011). In particular, the metabolic activity of ïĄğ-Pro was least affected by nanomolar PUAs, although those of Bacteroidetes and Rhodobacteraceae were markedly depressed (Balestra et al., 2011). Meanwhile, the daily addition of 1 nmolL-1 PUAs was found to not affect bacterial abundance and community composition during a mesocosm experiment in the Bothnian Sea (Paul et al., 2012)."

8. It is unclear why the authors studied the effect of polyunsaturated aldehydes of bacterial communities. The importance of polyunsaturated aldehydes was not properly and clearly presented. For examples, although the authors mentioned the effect of polyunsaturated aldehyde on marine microorganisms, detailed processes and mechanisms are not provided.

Response: We agree with the reviewer on this. In the revised manuscript, we have carefully rewritten this part by emphasizing the importance of PUAs on the microbial community and the associated mechanisms. " Phytoplankton-derived polyunsaturated aldehydes (PUAs) are known to affect marine microorganisms over various trophic levels by acting as infochemicals and/or chemical defenses (Ribalet et al., 2008; Ianora and Miralto, 2010; Edwards et al., 2015; Franzè et al., 2018). The strong effect of PUAs on bacterial growth, production, and respiration has been well demonstrated in laboratory studies (Ribalet et al., 2008) and in the field studies (Balestra et al., 2011; Edwards et al., 2015). A perennial bloom of PUA-producing diatoms in the PRE mouth (Wu and Li, 2016) should indicate the importance of PUAs for microbial activity here compared to many other organic compounds, such as 2-n-pentyl-4-quinolinol (Long et al., 2003) and acylated homoserine lactones (Hmelo et al., 2011). A nanomolar level of PUAs recently reported in the coastal waters outside the PRE was hypothesized to affect oxygen depletion by promoting microbial utilization of organic matters in the bottom waters (Wu and Li, 2016), while the actual role of PUAs on bacterial metabolism

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within the bottom hypoxia remains largely unexplored.".

9. Line 43: Please provide reference for the higher abundance of PAB compared to FLB.

Response: Done. The revised sentence is written as " In some coastal waters, PAB could be more abundant than the FLB with higher metabolic activity and may affect coastal carbon cycle through organic matter remineralization (Garneau et al., 2009; Lee et al., 2015)."

10. Line 59-60: How polyunsaturated aldehyde affects marine microorganisms? Please provide more details.

Response: Done. We have rewritten the sentence as " Phytoplankton-derived polyunsaturated aldehydes (PUAs) are known to affect marine microorganisms over various trophic levels by acting as infochemicals and/or chemical defenses (Ribalet et al., 2008; Ianora and Miralto, 2010; Edwards et al., 2015; Franzè et al., 2018). ".

11. Line 63: What is the meaning of affect oxygen depletion? Is this a promoting or inhibiting process?

Response: Done. The sentence has been rewritten as "... affect oxygen depletion by promoting microbial utilization of organic matters ...".

12. Line 72-73: It is strange to place this sentence here. Why PUAs did not serve as carbon source?

Response: Agree. We have moved the sentence to the method and result sections. Firstly, PUA can be toxic to some bacteria precluding its use as a carbon source. Secondly, the specific arrangement of two double bonds and carbonyl chain make PUA not a group of labile organic carbon for bacterial utilization. There were other studies supporting that PUA could not serve as a carbon source for bacterial growth (Ribalet., 2008; Edwards et al., 2015).

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13. Line 81: Is that “July 2nd”?

Response: It is July 2nd. We have corrected this typo in the revised manuscript.

14. Line 88: what do you mean by “pPUAs and dPUAs”?

Response: Done. The abbreviations of pPUAs and dPUAs have been defined in the revised manuscript.

15. Line 265: I did not see description of methods about the bacterial community analysis in bottom waters of X1, X2, X3 and PAB on particles of >25 μm.

Response: Done. We have added the method descriptions of these data to the section of 2.7.4 in the revised manuscript. " DNA samples for the bulk bacteria (>0.2 μm) and PAB on particles of > 25 μm at station Y1 were also collected for bacterial community analysis using the same method described above. Methods for the bulk water bacterial community analyses at station X1, X2, and X3 during the 2016 cruise can be found in the published paper of Xu et al. (2018).".

16. Line 276: Please provide the concentration of pPUAs and dPUAs.

Response: Done. The mean concentration of pPUAs and dPUAs has been provided in the revised manuscript.

17. Line 278: shown Line 391: According to the results, low dose (1 μmol) of PUAs can stimulate the growth of PAB, significantly different from that of high dose (100 μmol) treatment. However, the test of PUAs as organic carbon source was conducted with 200 μmol of PUAs. I guess such a high concentration would adversely affect bacteria growth, while the low dose PUAs is likely to be used as organic sources.

Response: The 200 μM PUAs used in the test of carbon source possibility was to assure the same level of organic carbon substrate as those for ALK and PAHs. Bacteria may need a longer time and a higher substrate concentration to utilize these refractory organic matters (ALK and PAHs). Although we have no test for the low-dose PUAs, the

previous study has suggested that low-level PUAs (1 μM and 10 μM) were not used as a carbon source by bacteria (Edwards et al., 2015).

18. Line 686: data of panel D are reproduced from Ribalet et al., 2008. Is this panel E? No methods were provided for growth of Alteromonas hispanica MOLA151.

Response: Agree. It should be panel E. We have corrected this in the revised manuscript. We have also provided the growth method of A. hispanica MOLA151.

19. Line 448: Since bacteria on >25 μm particles can be low, hypothesis on signaling molecules may be tuned down.

Response: Agree. We have rewritten this sentence as "... we hypothesize that PUAs may likely act as signaling molecules for coordination among the high-density PAB below the salt-wedge, which will potentially allow bacteria such as Alteromonas to thrive in degrading particulate organic matters ... ".

Please also note the supplement to this comment:
https://bg.copernicus.org/preprints/bg-2020-243/bg-2020-243-AC2-supplement.pdf

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