

## ***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.***

**Anonymous Referee #1**

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Review Wu et al 2020, bg-2020-243 Title\_ Impacts of biogenic polyunsaturated aldehydes on metabolism and community composition of particle-attached bacteria in coastal hypoxia. General comments The authors study poly-unsaturated aldehydes (PUA) and their influence on particle-attached bacterial abundance, taxonomy and activity. This is done in an partially hypoxic coastal area of the Pearl River Estuary during June in two consecutive years using 12 h to 30 d incubation. The authors conclude that PUA can influence bacterial abundance, taxonomy, growth and respiration. The manuscript is generally well written with proper language although some sentences need improve-

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ments. Results are well presented in the figures, but some complement of error bars are needed. I lack a convincing motivation to the importance of PUAs compared to the multitude of other organic compounds, and the importance of particle-attached bacteria as compared to free-living. No direct comparison with other organic compounds or free-living bacteria is done in the study. A general importance of PUAs and particle-attached bacteria should be tuned down in the discussion and conclusion. The effects of PUA on particle-attached bacteria is still of value as such. A short-coming of the experimental design is a lack of true replication of the treatments. In addition, the fact that only one season has been investigated. I also miss proper measurement of the abundance, acidity and taxonomy of free-living bacterial to put the claimed influence of particle attached bacteria in perspective. A similar argument for the lack of other organic compounds in the study. The conclusions must therefore be made more cautious, specific and these shortcomings commented on. Some speculative statements in the discussion and conclusion section need to be removed or rephrased. There are parts of the method descriptions that need to be clarified, better specified or added. A major revision in this spirit is required to motivate publication. Detailed comments  
r. 13-14 There is an extensive literature on eutrophication driven hypoxia in e.g. the Baltic Sea since 4 decades (cf. Cloern 2001). Please rephrase sentence accordingly  
r. 15. Do you mean “...water mainly dominated...”.  
r. 21 Please change “activity” to “...e.g. bacterial respiration and growth...”) and revise the sentence.  
r. 35 Change to “...deoxygenation is also tightly...”  
r. 43-45 In most aquatic environments free living bacteria are dominating in numbers as well as biomass (e.g. Kirchman 2008). The reference is not convincingly showing that particle-associated bacterial dominate in terms of abundance or how the growth of particle associated bacteria was measured. Please revise the message. This also question the focus on particle associated bacteria in the manuscript.  
r. 49-51 In many cases free-living bacteria dominate the respiration (Robinson and leB Williams 2005). Both types of bacteria is preferably studied. This question the general relevance of the study.  
r. 64-65. However, many other organic compounds may drive the bacterial respiration. Please provide some reference show-

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ing to that extent PUF is contributing to bacterial respiration. r.68-76 I would prefer more explicit research questions to be addressed in this paragraph for clarity and coupling to performed experiments. r. 84-85 Please define here what depths that were used for middle and bottom water categories (e.g. figure 6). r. 87-88 Please define the abbreviations pPUA and dPUA. r. 97 Filtration and freezing of nutrient samples may release nutrients from broken cells. r.124-129 How is the centrifugation and re-suspension of particles influencing their morphology, attachments of PUAs and PABs? r. 131-132 Please provide the recovery efficiency of particle attached PUAs after the preparation procedure described. r. 136-137. Please provide a reference where the method is validated. r. 146-147 Freeze thawing may relate PUAs from living cells also. r. 152 Should it be nmol per some volume or particle unit? r.160 Please specify what is meant by clean. What was the washing procedure? r.162 As presented here there was no true replication of the treatments? r. 167 How does methanol included in the procedure affect bacterial abundance and activity? Any control or test for this? Please comment on relevance for natural conditions. r. 176-179 Give some information on how close to natural conditions these final concentration of PUAs are. r.180 it is not obvious that turbidity will be detected if cells remain below about 109 cells cm<sup>3</sup>. Were the cell concentration measured by direct microscopy? r. 181 Please provide a description on incubation conditions and length. r. 185-196 The description of methodology is unclear. Please make clear if and how free-living bacterial abundance was measured? How is the methanol treatment accounting for bacteria from breaking particles? IN addition, please provide a reference validating this method. Give some measure of the precision of the flow cytometer analysis and a relevant reference. r.198-202 As no relevant pre-filtration is used you may include other organisms than bacteria in the respiration estimate. Please clarify and rephrase as needed. r. 208-209 What was the final TCS concentration in the sample. This does not follow the common procedure. Neither use of ethanol. r. 241 However, the lack of true replicates for the treatment (i.e. replicate 1-L Nalgene bottles per treatment) question a reliable result from the t-test. r. 251, Figure 1. Consider to reverse the colour palette. More logical to have blue for well oxygenated

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and red for hypoxia. Change “constant” to “similar”. r. 261-263. Please provide a statistical test for the claimed difference and confidence intervals (error bars) in figure 4. r.265. Provide statistical results for the claimed difference between bacterial phyla. Do the same for other differences claimed throughout the manuscript. r. 273 Should it be Bacteroidetes also here? Figure 5. Please present the type of error bars used. Same for all figures with error bars. r. 320-329 What can be considered significant differences as opposed to random variation in this analysis. Please motivate convincingly. r. 335 One month is an extremely long incubation. How relevant is this for the application to the natural environment? r. 360 Would be more informative to use a unit per particle or mass of particles? Litre of particles is unclear. r. 365 This assumes that PUA is a major substrate among all other organic compounds. Please provide some references on this matter and discuss it critically. r. 368 This should be compared with the biomass of free living bacteria. They may also be elevated in the hypoxic water. I find the lack of measurement of free-living bacteria a short coming in the context of claiming importance of PABs. r. 372-373. How is respiration by particle-attached bacteria distinguished from protozoa, phytoplankton and larger zooplankton? This is typically difficult to achieve. Comment in a critical manner. r. 376-390. Given the apparent lack of true replication of the treatments (i.e. replicate 1 L Nalgene bottles) the conclusions regarding treatment effects is highly uncertain. This needs a discussion. r. 389-390 Relevance in the natural environment assumes that the applied concentrations are relevant for those occurring in the natural environment. Please consider, discuss and modify the conclusion accordingly. r. 391-392 I find it valuable to know if PUA stimulates bacterial activity whether as an organic substrate or metabolic signal substance. Please explain why only the latter would be ecologically important. r. 392-394 Please use the same concentration unit for comparability of levels. Heptadienal alone used for the test may not be comparable to a mixture of different PUAs (i.e. concentration more than twice used in the combined concentration). Why was not the same mixture used for this experiment? Other methods like using labelled PUA and analyse for metabolism of those would better test the mechanism of PUA effect. r. 405-407 Please consider that a few

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species within the *Alteromonas* phylum may be responsible for the observed response. PUA metabolism might not be a function attributed to the whole phyla. Rephrase the discussion accordingly. r. 411-412 Please refer to what figure and test that show a difference between particle-attached and bulk bacteria. r. 427 How strongly are PUAs adsorbed to particles (i.e. chemical bonding)? How may this influence their potential to act as signalling molecules? r. 439-441 I have not seen any analyses of the lipoxygenase hypothesis in the study? It is thus speculative and should be removed. Focus on conclusion that can be derived from the performed study. r. 442-445 As there was no true replicates this conclusion should be made more cautious. r.455-460 If this section should remain it need to be moved to the discussion section. r. 460-464 The sudden appearance of PUFA is not connected to the previous sentence?. Again, this part is highly speculative and not part of conclusion from the study. Remove or move parts to the discussion. Literature cited Cloern, J. E. 2001. Our evolving conceptual model of the coastal eutrophication problem. *Mar. Ecol.-Prog. Ser.* 210: 223-253, doi. Robinson, C., and P. J. Le B Williams. 2005. Respiration and it's measurement in surface marine waters, p. 147-180. In P. A. del Giorgio and P. J. Williams, le B [eds.], *Respiration in aquatic ecosystems*. Oxford University Press.

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