

Interactive comment on “Trace chemical species in marine incubation experiments, part A. Experiment design and bacterial abundance control extracellular H₂O₂ concentrations” by Mark J. Hopwood et al.

Ma (Referee)

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This work provides large scale mesocosm experiments to elucidate how microbial groups affect extracellular H₂O₂ concentrations and other related questions. It has shown that the high bacterial densities were associated with low H₂O₂.

This manuscript generally reads well and presents a good rationale of research. However, the study could be significantly improved with the addition of missing details on the methodology used in experiment design, as well as statistical support.

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The major issue is that there are so many variables in this work, which have not been fully considered regarding to the result interpretation. All these variables could play a great role in affecting the extracellular H₂O₂ concentration while the rationale to use these variables were not explained well and when the conclusion could not be obtained between microbial groups and H₂O₂ concentrations if all other variables were playing great role in it. These variables include (not limited to): zooplankton concentrations, different bacterial community, temperature, nutrient (concentrations and chemicals), light (light cycle and light intensity), DOC and pH. For example: In Glippa et al., 2018, "Vehmaa et al. [21] found that a 3 degrees rise in temperature increased the antioxidant capacity (ORAC, Oxygen Reactive Absorbance Capacity) in *Acartia* copepods by almost 15%, and they measured a 2-fold increase also in oxidative damage, measured as lipid peroxidation".

Specific comments:

The line numbers started over on each page. It is better to have continuous line number from the beginning to the end of the manuscript.

P9 L27: Is there statistics to support the "H₂O₂ was generally elevated"?

P11 L9-L10: It is hard to get the conclusion of "this trend closely matched that observed in zooplankton biomass" by only eyeballing it, especially when the 5th day of zooplankton biomass was not shown in the figure.

P12 L13: Statistics would be helpful to support "a clear difference was noted between".

P13 L7-L8: Again statistics would be needed to the statement "there was a more pronounced increase".

P13 L1-L13: Regarding to the statements, "In the low pH treatment (initially 7.54 ± 0.09), H₂O₂ concentrations were significantly higher (Mann-Whitney Rank Sum test p 0.02) compared to the unmodified pH treatment (initially 8.01 ± 0.02)". Only by eye-balling it, it showed the LG0.5C LpH and LG 1C LpH have higher concentration

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of H₂O₂. Is this statement based on only these two data points? Regarding to the statistics p value, it would be helpful if it is equal to, less than or greater than some certain number by indicating with corresponding symbols.

P15 L8-L13: It would be great to put these discussions after (Table 1) under Discussion.

P16 L16-L17: Regarding to this statement, “Bacterial production showed no statistically significant (ANOVA, P 0.562) difference between low, medium and high H₂O₂ treatments.”, there is no data to support it. Is it related with Fig. 9(c)?

P17 L3: The author claimed there is NO significant difference while the p value is less than 0.05.

Figure 1: There is line to indicate the Mean H₂O₂. However, it is not clear on how to get this Mean.

Figure 2: Is there any interpretation on the big variation of H₂O₂ in ambient? Is there replicates to have error bar? Statistics would be helpful here to show the difference between HG/LG status.

Figure 7: It would be great to show diurnal cycling of H₂O₂ in two continuous days.

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