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Biogeosciences Discussions

Interactive comment on "Trace chemical species in marine incubation experiments, part A. Experiment design and bacterial abundance control extracellular H_2O_2 concentrations" *by* Mark J. Hopwood et al.

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Two reviewers are thanked for insightful comments on the submitted text. We respond to all of Dr Ma's comments here (some of which were posted earlier as a 'comment' rather than a 'review').

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. 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".

Reply: There are of course many variables which exert influence on extracellular H2O2 concentrations. One the main rationale for working with mesocosm experiments was that intra-experiment data is free from variation in some of these variables. Salinity/temperature/light exposure/nutrient addition are close to constant across the mesocosm units within each experiment. We have added a paragraph to explain this rationale (below). Concerning between-experiment differences, these are of course more challenging to explain because there are differences in physical/biogeochemical parameters between fieldsites. This is a main reason why we attempted to 'normalize' data to ambient H2O2 concentrations as this (and some tests on our experiment setup) provides the strongest evidence that low H2O2 across many of the experiments arises simply from the plastic containers used rather than 'natural' parameters. "....our rationale for the investigation of H2O2 trends during these 20-8000 L scale mesocosm and microcosm experiments is that the experiment matrixes for each experiment permitted the changing of 1,2 or 3 key variables (DOC, zooplankton, pH) whilst maintain others (e.g. salinity, temperature, light) in a constant state across the mesocosm/microcosm

experiment. The relationships between H2O2 and other chemical/biological parameters are therefore potentially easier to investigate than in the ambient water column where mixing and the vertical/lateral trends in H2O2 concentrations must also be considered. Additionally, two of the experiment designs described herein (see Table 1) were repeated in 3 geographic locations facilitating direct comparisons between the experiment results with only limited mitigating factors concerning method changes."

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.

Reply: Changed in Revised text.

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

Reply: A line is now added in the revised text. In this particular case, the difference was so large we didn't think it necessary to detail ANOVA results, the mean/median ambient level is at least 40% lower than any treatment.

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.

Reply: a reason why there is no statistical test here is because, for logistical reasons which we acknowledge are not ideal, the zooplankton biomass data and the H2O2 data are at different timepoints. There isn't a 'missing' datapoint, there is simply a lower resolution for zooplankton data in this experiment and a temporal mismatch between the two data series. One of the experimental problems, which we raise in the text already is that any inter-day temporal trend in [H2O2] made using 'spot' measurements must be done at the same time daily. Where possible (and basically wherever there are stats present in the manuscript), we timed the measurement of all parameters to be the same so that we can directly compare [H2O2] to other parameters and report [H2O2] at the same time daily. However, for some parameters, including zooplankton

during MesoMed, such a coherent timing simply wasn't possible due to the significant amount of time required to sample these parameters from the mesocosms. In these experiments, where we can only comment on the general trend, we have rephrased the text to highlight the uncertainty. The line referred to (P11 L9-10) is removed.

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

Reply: t test added comparing the two groups ($p < 0.001$) accordingly.

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

Reply: regression/standard error details added (HG 0.31 ± 0.1, LG 1.2 ± 0.1) accordingly.

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 eyeballing it, it showed the LG0.5C LpH and LG 1C LpH have higher concentration 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.

Reply: P values are now labelled < / > / = . Yes there are two very high H2O2 values in this dataset, both of which happen to be low pH/medium carbon treatments. If these values are excluded then the significance of the difference between low pH and high pH treatments disappears. Whilst there are only a limited number of datapoints in each (low/high) pH category, these two can be defined as anomalies based on 1.5 IQR if we look at the low pH and normal pH sets as groups of 8. This is now noted in the text.

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

Reply: amended.

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)?

Reply: No this is a separate side experiment. We had included a figure to show these data but dropped it to save space. The values (triplicate $\pm$ SD) are now provided within the text. . . “Bacterial production showed no statistically significant (ANOVA, p=0.562) difference between triplicate low (1.69 $\pm$ 0.28 μg C L-1 day-1), medium (1.30 $\pm$ 0.60 μg C L-1 day-1) and high (1.29 $\pm$ 0.56 μg C L-1 day-1) H2O2 treatments”

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

Reply: Typo corrected, should have been ‘> 0.05’ not ‘< 0.05’

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

Reply: Clarified in the figure label. . .. “Data from Hopwood et al., (2018). The mean ($\pm$ SD) H2O2 from all 8 pCO2 treatments is shown”

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.

Reply: We can of course speculate. The ‘ambient’ measurements always refer to the coastal ocean. Unlike the other fieldsites (Svalbard, Patagonia, Gran Canaria), this location (for the Mediterranean/Crete experiments) was not a sheltered fjord or harbor which likely means the H2O2 is much more variable due to changing stratification in the water column. But as we only sampled surface water at intervals during the experiment we can’t really quantify this or do anything other than speculate about the underlying causes.

The discussion of the zooplankton trend is now not explicitly linked to H2O2 (see com-

ment above). Noting the different timing of the measurements during this specific experiment it is not possible to produce meaningful statistics.

There are replicate measurements for all ambient water measurements, which produce a very small error bar (1-5%). However, given the short-term changes to H2O2 that can occur in a dynamic water column even on very short (minutes) timescales (as demonstrated in our high resolution diurnal time series) we thought that plotting error bars based on analytical error for spot measurements would be misleading as it is not inclusive of the changes to [H2O2] that occur in natural waters over a time period equivalent to the sample collection/measurement time of 10-20 minutes.

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

Reply: It would, but when the apparatus is set up to produce continuous data like this an analyst has to check on the instruments very regularly. It simply wasn’t possible here to have them operating for more than 24 hours! We may try a different instrument/sensor configuration to achieve this in the future with slightly lower resolution and an auto-clean cycle.

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