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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.

Anonymous Referee #1

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The goal of this study was to determine if aspects of an experimental design could inadvertently affect the photochemical or biological production of hydrogen peroxide (H2O2), thus altering the outcome of the study. This was tested by analyzing the compiled data from multiple coastal mesocosm experiments and determining which factors or aspects of the experimental design caused a change in H2O2 concentration compared to the ambient concentration found in surrounding seawater. Based upon their analysis, the authors concluded that the isolation of seawater within a mesocosm, alterations to light intensity, and changes to bacterial abundance were responsible for

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variations in H2O2 concentration between the mesocosm vessels and the surrounding seawater. This study represents an interesting opportunity to observe how standard methods of experimental design (mesocosms) could potentially influence experimental outcomes in marine environments. Additionally, this study is unique in how the authors explore the effect of organisms of higher trophic levels upon H2O2 concentrations. The authors were able to provide convincing evidence supporting the importance of bacterial communities in modulating H2O2 concentrations in the ocean.

Major comments: A major conclusion of the paper is that light treatment (ambient versus artificial) has a big impact on the H2O2 concentrations in the mesocosm experiment. While this is supported by the figures, it is difficult to tell which light treatments are used for each figure, and there is no indication in Table 1 if the mesocosms are exposed to sunlight or light bulbs. Along these lines, there is essentially no discussion of the differences in light exposure, particularly the ability of UV in sunlight to generate the H2O2, and this should be mentioned in both the introduction and the discussion. The authors attempt to demonstrate how aspects of an experimental design (structure of vessel, setup, nutrient addition, increased stress) could affect the concentration of H2O2. While changes in H2O2 are measurable in all mesocosm experiments and are potentially attributable to a particular aspect of the experiment, the observed changes in H2O2 concentration are small with respect to total daily production of H2O2. All but one of the mesocosm experiments have H2O2 concentrations below 100nM and ranges of variation between 20-50nM. The prospect of changes in H2O2 concentration such as these recorded altering experimental outcome for microbial activity and DOC decay seems unlikely, without cited support. Pg. 18 lines 24-26 – As stated here, no clear trends can be defined between H2O2 concentration and grazer abundance when considering all datasets used. Perhaps it would be beneficial to focus more intently upon the aspect of bacterial abundance and its effect upon H2O2 concentrations instead? Along with above comment, bacterial abundance is an integral part of this study's conclusions yet only 2 figures give any data on how their abundances are changing. Inclusion of cells count data for the other experiments and datasets would

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strengthen this major argument of the paper.

Minor comments:

The authors claim that the isolation of seawater in mesocosm vessels allows for the accumulation of H2O2. This is discussed throughout the manuscript but notably in Figure 1. on pg. 9 line 22-32 and pg. 21 line 1-11. In Figure 1, the authors claim that there is no clear trend between H2O2 and pCO2 concentration, leading them to conclude that changes in H2O2 are due to the enclosure used to house the water. Does this graph show H2O2 concentrations in unamended seawater within one of the polyurethane bags used, i.e. is the baseline 400atm a control? If not, then H2O2 production cannot solely be attributed to the container used. In Figure 1 is it possible that the microbes are nutrient depleted by day 8-9, and the increase in H2O2 is due to their decline in abundance? This would also explain why the H2O2 concentration decreases around day 18 when the nutrient addition was made. Axis labels throughout manuscript are misleading. H2O2 / nM should be shown as H2O2 (nM), etc. In Figure 2 panel a, the H2O2 concentrations for ambient seawater and LG 2C treatment are difficult to discern. Consider a different representation of the data. Pg. 20 lines 15-20 – The authors are comparing H2O2 production ranges from open ocean environments to those measured in coastal environments. In Table 2 on pg. 20, the upper H2O2 concentrations listed for the Crete and Patagonia locations are significantly higher than any data shown in previous figures from those same locations. Pg. 21 lines 13-14 – Were individual microbial groups ever quantified? Or was this observation made from total cell counts? Figures 4a and 5a: are these data from the same experiment? The values for “LG 1C” look different in these figures, as one example.

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