

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.

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Received and published: 20 December 2018

Two reviewers are thanked for insightful comments on the submitted text.

Please note that in addition to the comments by reviewers on this text, a companion manuscript concerning a different aspect of the same mesocosm experiments was also recently reviewed for this journal. As it is highly desirable to have a consistent use of terminology between these (and other in prep.) texts concerning the experiment set up, the following change has also been made to this text in order to maintain consis-

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tency: The names of the major experiments has been standardized throughout the text and we have been careful to use only one specific term of reference for each experiment. The mesocosm/microcosm/multistressor experiments are now termed MesoPat/MesoArc/MesoMed/MultiPat/MultiArc/MultiMed/MicroPat/Gran Canaria.

(Previously the term 'MesoPat' was used to loosely refer to the field campaign which included a trio of mesocosm/multistressor/microcosm experiments, but this was found to be confusing, 'MesoPat' now refers exclusively to the 1000 L scale mesocosm experiment conducted in Patagonia, 'MultiPat' to the 20 L multistressor experiment at the same fieldsite location etc...).

Anonymous Referee #1 Received and published: 24 July 2018 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 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

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are used for each figure, and there is no indication in Table 1 if the mesocosms are exposed to sunlight or light bulbs.

Reply: We have made this important clarification throughout the text. Extra lines are added in Table 1 to state the exact light 'setup' for each experiment and within the text we have clarified which experiments were outdoor/indoor lighting arrangements.

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.

Reply: Information is added to the introduction to briefly outline the concept, "Quantum yields for H₂O₂ formation increase with declining wavelength and so the ultraviolet (UV) portion of natural sunlight is a major source of H₂O₂ in surface aquatic environments (Cooper et al., 1988, 1994). Sunlight normalized H₂O₂ production rates therefore peak between wavelengths of 310-340 nm (Kieber et al., 2014)." Additionally, we further add a description of the lighting different and the ability of HDPE to remove/reduce UV light in the discussion, "...considering the light arrangements for these experiments (Table 1). The Gran Canaria experiment was practically unshaded with surface seawater exposed to natural sunlight. The closed HDPE mesocosms (MesoMed, MesoPat, MesoArc) experienced natural sunlight but after attenuation through 1-2 cm of HDPE plastic. Whilst the transmission of different light wavelengths through these HDPE containers was not tested during our experiments, 1-2 cm of polyethylene should strongly attenuate the UV component of sunlight. The 20 L scale experiments (MultiMed, MultiPat, MultiArc and MicroPat) were conducted using identical synthetic lighting with lamps selected to as closely as possible replicate the wavelength distribution of natural sunlight. However, the fluorescent light distribution is still deficient, relative to sunlight, in wavelengths <400 nm, which is the main fraction of light that drives H₂O₂ formation in surface seawater (Kieber et al., 2014), and these containers still mitigated the limited UV exposure with a 1 mm HDPE layer which would further reduce the UV component of incoming light..."

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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.

Reply: These changes are certainly small and it is doubtful that the variation between different treatments within the mesocosms/multistressor experiments had measurable effects. However the side experiment in Gran Canaria did suggest a positive effect on bacteria when water was subject to a H₂O₂ decline equivalent to the 'gap' between natural and incubated water during some of these experiments. Nevertheless, we acknowledge that diurnal changes in H₂O₂ are large, and this large variation complicates any data interpretation about temporal changes in daily mean H₂O₂. This is now explicitly stated in the text, "A specific challenge with determining the effect(s) of H₂O₂ concentration on any biogeochemical processes, and vice-versa, is that the diurnal variability in H₂O₂ concentration is always large compared to inter-treatment differences in H₂O₂ concentration within individual experiments (e.g. Fig. 11)..."

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 strengthen this major argument of the paper.

Reply: This is perhaps clear after we present the data. The logic behind a focus on zoo-

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plankton/pH/DOC was that these were gradients which were present in all experiments that could [we thought] plausibly affect equilibrium H₂O₂ concentrations. It wasn't clear until after looking at the data that no clear effect of zooplankton (or pH) on H₂O₂ was evident. We presently show bacterial productivity data for all experiments and are not sure that it is necessary to plot cell counts and productivity separately in addition to the synthesis of all data (Fig. 10). In the case of bacteria as a H₂O₂ sink, an additional complication is the very low H₂O₂ concentrations at the end of all MultiPat/Arc/Med experiments which makes it challenging to find changes in [H₂O₂] due to the reduced signal:noise ratio. More importantly, there is also a biological issue here (which we now mention in the text – our discussion concerning the role of bacteria (s 4.1) is expanded), because microbial organisms may adapt the strength of their oxidative defenses to ambient H₂O₂ concentrations i.e. cellular H₂O₂ defences are less active at lower H₂O₂ concentrations. Even for those experiments where detailed counts (total, or species level), are available, it therefore becomes difficult to make any valid argument concerning cell counts and group/species level abundances at these low H₂O₂ concentrations as the relationship between cell counts and H₂O₂ concentration would only likely be observed at higher H₂O₂ concentrations. “the H₂O₂-defence mechanism of organisms may also be sensitive to ambient H₂O₂ concentrations. Morris et al., (2016) suggest that microbial communities exposed to high H₂O₂ have elevated H₂O₂ defences. If the microbial communities here exhibited a dynamic response to H₂O₂ concentrations in terms of their extracellular H₂O₂ removal rates, this would dampen the correlation between bacterial abundance and H₂O₂ concentrations- especially at low H₂O₂ concentrations. ...”

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

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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.

Reply: for the experiment shown in Figure 1, yes the 400 atm 'treatment' is a control in this sense i.e. atmospheric PCO₂ with no additions of CO₂ made (and no other additions of any kind before the nutrient spike on day 18).

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.

Reply: amended accordingly.

Pg. 20 lines 15-20 – The authors are comparing H₂O₂ production ranges from open ocean environments to those measured in coastal environments.

Reply: This is now explicitly stated in the text, but does not really affect our interpretation. The key point was that some diurnal ranges in mesocosms are very high (higher than expected based on diurnal ranges in the same location) whereas some diurnal ranges in mesocosms are very low (based on diurnal ranges in the same location). The offshore values are shown for comparison only to help interpret the data.

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.

Reply: These refer to 'natural' seawater outside the experiments and are included for reference only to compare to the experimental results. This further clarified both in the text and in the abstract to avoid confusion.

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Pg. 21 lines 13-14 – Were individual microbial groups ever quantified? Or was this observation made from total cell counts?

Reply: For these experiments groups were quantified.

Figures 4a and 5a: are these data from the same experiment? The values for “LG 1C” look different in these figures, as one example.

Reply: No they are different datasets. 4(a) is MultiMed. 5(a) is MultiPat. We have reformatted the figure descriptions to highlight the experiment names better and avoid confusion.

Interactive comment on Biogeosciences Discuss., <https://doi.org/10.5194/bg-2018-247>, 2018.