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Interactive comment on "Release of hydrogen peroxide and antioxidant by the coral *Stylophora pistillata* to its external *milieu*" *by* R. Armoza-Zvuloni and Y. Shaked

Anonymous Referee #2

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General comments

In the proposed work, the authors present a new method and approach to calculate the kinetics of both the release and the degradation of H202 in the seawater surrounding coral nubbins. This methodology paper could have important implications in the field of coral physio-ecological responses, although some of the presented data have already been published in 2013 by the same authors (H2O2 and Kantiox in Stylophora sp.). I understand this paper is focused on the methodology and, hopefully, in a near future, other reports would be presented about effects of different stressors. Nevertheless, carrying out few complementary experiments should strengthen the scientific significance of the presented work. In addition, as listed below, several precisions in

the experimental design are needed to reinforce the scientific contribution of this new methodology, which provide kinetics parameters in part deduced from calculations.

Specific comments

#1: I have a first concern about the term “antioxidant” used throughout the article (including in the title). Although the authors try to make a -very basic- characterization of this so-called antioxidant activity, the nature of the involved compounds is not known yet. In fact, it is probably a mix of very different molecules released by either the coral nubbin or associated microorganisms, and the observed H2O2 decrease could be due -at least in part- to the conversion of H2O2 to much more oxidative molecules (in particular hydroxyl radicals). That is not just a play on words, and I would recommend to use a more neutral term like “H2O2 degradation” rather than “antioxidant”. This point (pro-oxidative conversion) should also be evoked when discussing about the nature of the possible “antioxidant” molecules.

#2: On this same aspect, if the authors really suspect some extracellular catalase release (very interesting point), why have they not used a specific inhibitor like 3-amino-1,2,4-triazole? This sounds much more relevant than a 80°C incubation! What’s about the time-course variation in the seawater protein content (as a proxi of mucus production) and correlation with stirring speed?

3# All measurements and calculations should be presented with their respective controls: seawater incubated under same conditions without coral nubbins! This was the case in the 2013 paper, why not here? This is absolutely necessary to assess the implication of both metallic (Fe, Cu, Al etc.) and organic contaminants (including microorganisms) present in the "natural seawater".

4# It is very frustrating that the presented data are only obtained under dim light and that there is no comparison between photoactive (bight light) and non photoactive (dim light) coral nubbins. The bleached vs symbiotic state comparison is very interesting and relevant, but this does not solve out what is going on in a photoactive symbiotic

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coral! This very straightforward experiment (ligh/dark cycle) would help the reader to get convinced about the validity of the methodology, in a much more relevant manner than with the effects of variable stirring speeds!

5# The authors should consider deleting the fig 5, which bring the exact same data as fig 4.

6# In the text (lines 9-10 pg 40) it is explained that “Kantiox increases linearly with time” and that Kantiox value is then calculated “using a linear equation that was fitted through the data”, but no data are further presented in the result session to show this correlation. I tried to make some calculations from the slopes presented fig 1d, and I found an r2 of 0.91 for a linear and an r2 of 0.97 for an exponential correlation! If fig-1 results are representative, kantiox seems to increase exponentially and not linearly, which has major consequences considering long incubation times (400 min as in Fig-3). This point really needs clarifications by showing the mean variations with standard errors and corresponding statistical test of linearity (or exp.) and not just few points of one experiment, as it is the case Fig-2.

7# Why the Kantiox and H202 variations over time presented in the Fig-2 are so different (40 times higher), than the one published previously (Fig 8 of the 2013 paper)? Is it due to stylophora species or just to a coral size/beaker volume ratio? If size/volume ratio is correct, that means that the indicated values should really be normalized by the relevant factor to allow further comparisons. Similarly, to me it is necessary in such a methodology paper to verify that the kinetics parameters are well correlated to the nubbin size/surface. The authors should give indications about the min/max limits in the biological material amount needed to achieve relevant measurements.

8# Many method precisions should be given in the changing flow experiments: - slow/moderate/fast are not scientifically relevant terms if not connected to values expressed either in rpm or even better in water movement speed. - since the same corral nubbin is consecutively submitted to the three flows, the statistical test should be a

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repeated measures ANOVA (at least if ANOVA test conditions are respected... which I doubt concerning the homoscedasticity). - at the highest speed, did the vortex formation created some foaming, which could probably interfere with the observed results? - since coral nubbins were suspended by a Nylon thread, the fragments should also rotate due to stirring, modifying the relative flow to which the coral surface was actually submitted in the beaker. - since these experiments are done in such a small volume (100ml), without water renewal, authors should not compare their findings to the effects of open-sea currents (line 6, pg 49). - Are the observed effects reversible with further decreasing speeds?

9# Although I understand the need of it for calculation, presentation of data corresponding to exogenous H202 addition in the bleached coral is very confusing (Fig 4-d).

10# In the method session we should know how long is the incubation in heating water bath (is it 10 min. as for azide?). The end of this paragraph (lines 24-25 pg 40) is very confusing, since no further data are presented about these “highly active samples”.

Technical corrections

In the Fig-6, the half of the H2O2 concentration figures is not visible.

Interactive comment on Biogeosciences Discuss., 11, 33, 2014.