

## ***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 #1**

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This is the second time I have reviewed this manuscript that in its original form was combined with Shaked and Armoza-Zvuloni (2013). Having read the published part of that contribution and this submission to BGD I find very few, if any, of my original comments were incorporated or rebutted. Most of my concern then, and now, revolves around the Materials and Methods. They are poorly described and render the experiments largely unrepeatably. Additionally, the design that one can decipher strongly suggests that the statistics applied are incorrect making an evaluation of the results and their interpretation in the Discussion difficult to do. As a result I will confine most, but not all, of my comments to the M+M. First and foremost the manuscript is poorly organized making it difficult to read, as was the original combined manuscript.

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## Abstract

P 34, line 17; Please define “ventilation”. There is no term that I’m aware of in the hydrodynamic/fluid dynamic literature that uses the term “ventilation”. One assumes the authors are possibly talking about changes in the diffusive boundary layer with flow and subsequent mass flux changes but the readers are left to wonder what the intent of the authors is here.

## Introduction

P 35, line 18; ROS production, largely as a result of hyperoxia, in symbiotic cnidarians has been well known for a long time. The authors references don’t reflect that history. P 35, line 19; Here again, using Papina et al. 2003 as a primary reference to describe the nature of the coral algal symbiosis is problematic. What about Muscatine, Dubinsky, Porter Falkowski and others who actually did the original work???

## Materials and Methods

P 37, line 4; How many fragments?

P 37, line 6; How long is “a short period” for acclimating?

P37, line 8; Water is not “homogenized”, it is mixed.

P 37, lines 13-14; The issue of keeping the volume constant in the incubation seawater seems to me to require some sort of dilution factor. You are adding fresh seawater to the incubation and that would dilute any measured constituent, however slightly, in the medium.

P 37, lines 17-18; Irradiance should be expressed as  $\mu\text{mole quanta m}^{-2} \text{ s}^{-1}$  which are SI units.

P 37, So it is unclear from this initial description how many replicate corals were used; you have multiple factors in play including time, flow speed, and light versus dark and the analysis of these experiments later in the paper doesn’t discuss any interactions

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between the three. The experimental design is completely unclear and as written the analysis appears to be completely inappropriate. Also, later in the paper it appears that multiple experiments were done over a period of a year. The authors have combined these data for analysis but they should be blocked for time to see if the results of experiments done at different times, even under the same supposed conditions, produced different results.

P 38. In my previous review I raised the issue that both POHPPA and catalase are light sensitive. While the authors now say the samples being tested were kept in the dark my concerns were about the stock reagents. Also, and again from my previous review, no metal chelating agents (e.g., DTPA) were used in these assays/experiments. The authors do understand the importance of chelating redox metals as evidenced from their previous publications but nowhere have those same precautions been taken in this work that this reviewer finds worrisome.

#### Discussion

I can make no substantive comments because I do not know if any of the experiments were analyzed correctly.

P 47, lines 25-28 +; The notion that there would be a “pool” of hydrogen peroxide, in the absence of any direct evidence, defies our current knowledge of ROS biochemistry. It would be extremely dangerous for any cell to maintain a “pool” of hydrogen peroxide as if it isn’t broken down enzymatically can cause damage itself such as DNA damage and it is very susceptible to reduction to more toxic species of ROS especially if any transition metals (e.g., Fe) are available where Fenton chemistry could occur. The source of the hydrogen peroxide produced is likely mitochondrial in origin.

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

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