

## Review of the paper "Transcriptomic responses of sponge holobionts to *in situ*, seasonal anoxia and hypoxia" by Brian Strehlow et al.

This manuscript presents a study of the transcriptomic response of two sponge species to hypoxic and anoxic conditions *in situ*. The study focuses not only on the transcriptomic response of the sponges but also (very novel!) on that of their mitochondria and dominant symbionts. The manuscript is well written and the metadata, data analysis pipelines and scripts are available in an open repository, following good open-science practices.

This study deals with a very important and timely subject in the current context of climate change, in which benthic communities and ocean ecosystems in general are predicted to suffer oxygen limited. The authors found very interesting results, such as some potential gene functions for oxygen homeostasis in sponges (e.g., Hsp90) or the potential role of *Thaumarchaeota* symbionts in O<sub>2</sub> limiting conditions, being a source of O<sub>2</sub> for the sponge when O<sub>2</sub> is limiting.

This work has already undergone a review stage in which the authors have made an effort to simplify the presentation of their results and help the reader to get the take-home messages of their work. I still believe that the manuscript could be improved to highlight the most relevant results (see below my comment on this subject in the General comments).

Please find below some comments on general aspects of the manuscript and a series of detailed remarks on specific points.

### General comments

- In the Introduction, the authors cite a number of studies on the effect of hypoxia/anoxia on sponges. I suggest avoiding the use of zoological nomenclature to refer to each sponge species mentioned to improve the readability of the article. Ex. *Polymastia crocea* rather than *Polymastia crocea* Kelly-Borges and Bergquist, 1997
- Experimental design is complex (2 species, 3 O<sub>2</sub> conditions, ...) as is the data analysis (transcriptomics on sponge, mitochondria, symbionts) and the results explanation (pairwise conditions comparison, etc.). Replication also varies between species and conditions, limiting some comparatives (and eventually limiting data interpretation, which authors have been cautious in discussing, which I appreciate). I suggest adding a section or paragraph on the sampling strategy to help readers understand the experimental design and the results interpretation.
- State in the Methods which O<sub>2</sub> conditions have limited replicates (i.e., n < 3). This limitation in replicates may be responsible of some of your non-significant results.
- As above mention, the complex experimental design and the pairwise comparison of the conditions evaluated make complicated to identify the functional genes affected by O<sub>2</sub> limitation. In the Results, please not only indicate the up- or downregulated genes but also the functions affected. See my comment below as well.
- I know the authors have already done a big job making Figure 4, but I still think a figure or summary table showing the biological processes (e.g., energy metabolism, DNA repair, etc) affected by hypoxia, anoxia and deoxygenation for each species is necessary. Figure 4 illustrates differences between species (including mitochondria and symbionts) and O<sub>2</sub> conditions, but it does not show the differences of functions expressed to cope to or as consequence of limitation of O<sub>2</sub>. In this new figure/table, you could specify if the function has changed and if it is provided by the symbiont or it happened in the sponge itself.

## Detailed comments

L 158: change "...in sponge *Thaumarchaeota*" to "...in sponges with *Thaumarchaeota*", to "...in sponge symbionts *Thaumarchaeota*" or an alternative to make it clear.

L173-174: change "low atmospheric oxygen concentrations" to "low environmental oxygen concentrations".

L 182-185: indicate how you measured *in situ* oxygen conditions

L 194: add "Metadata for individual samples, including **sample code**, oxygen concentration, ... are included in Supplemental Table 1". These codes are used afterwards (e.g., L202) and it is not clear where they came from.

L 195-197: were these species identified based on their skeletons? Please, specify.

L 202: add "sample" before DC24, otherwise this code may not make sense to readers who do not read the Supplemental Information.

L 223: specify what the acronym SDU means

L 243: correct to "... generated  $2.05 \times 10^8$  and reads  $3.45 \times 10^8$  reads for..."

L 371 & 372: write the full name of *Eurypon* sp. 2 rather than *E. sp. 2*.

L 422-424: suggestion to simplify as "Expression patterns of both species were only similar in one case. KOG expression in *H. stellifera* under hypoxia versus anoxia significantly positively correlated with that of *Eurypon* sp. 2 under the same conditions ( $r = 0.43$ ,  $p < 0.05$ , Figure 1E)."

L 450-452: I suggest deleting this sentence as it is repetitive with figure legend and does not provide new relevant information.

L 456: "...depending on oxygen **availability**" or "... on the oxygen **level**".

L 498: given the nature of the study, the text is full of acronyms and abbreviations, so I suggest avoiding those not absolutely necessary, as for example DEGs.

L 507: delete "of the same", so sentence read "Upregulated genes included all genes that were significantly upregulated in anoxia"

L 511: change to "It is noteworthy, however, that all genes upregulated genes in anoxia..."

Figure 2 & 3: I suggest to write above the heatmaps the sponge species to which they belong, that is, *Eurypon* sp 2 for left heatmaps and *H. stellifera* for right ones.

L 669-674: write the full name of *Eurypon* sp. 2 rather than *E. sp. 2*. Italicize *H. stellifera* in L 671.

L 686: cite Table 1

L 777-779: change to "Sponges under heat stress also upregulate Hsp70 (López-Legentil et al. 2008; Guzman and Conaco 2016; Webster et al. 2013) and Hsp90 (Guzman and Conaco 2016), **as it occurred in both Lough Hyne sponges under hypoxia**"

L 786: anoxia vs hypoxia, doesn't it?

L 815: correct superoxide formula to  $O_2^{-2}$

L 901: correct the typo to *S. mosellana*

L 996: change to "AMO genes" rather than "amo genes"

L 1037: no need of italics

L 1076: write the full name of *Eurypon* sp. 2 rather than *E. sp. 2*.