General comments

The article by Strehlow et al., aims to investigate the functional effect of *in situ* deoxygenation on two sponge holobionts (*Eurypon* sp. 2 and *Hymeraphia stellifera* and their microbiome) using a transcriptomics approach. This study provides unique outcomes for the research field on sponge holobionts, helping to better understand their metabolic adaptation under hypoxia and anoxia, especially in an environmental context which could be relevant in for future oceanic conditions. The research conducted in this paper constitutes a logical complementary approach following Schuster et al. (2021), dedicated to the study of the microbiome composition from the same sampling set from Lough Huyne (Ireland). Authors provided informations on the up- and down-regulated functions changing from normoxia to hypoxia, normoxia to anoxia, and hypoxia to anoxia, not only for the sponge transcriptome, but also from transcriptomes of the sponge mitochondria, and two symbionts members discussed in Schuster et al., (2021) affiliated to *Thaumarchaeota* and *Gammaproteobacteria.* Through an extensive effort to describe all the functional differences observed, and integrating other studies (as a meta-analysis), the authors also highlighted the species-specific nature of the response to the deoxygenation. In overall the article is well written and the discussion is nicely structured.

Considering these reasons, I think that the paper has a great potential to have a good impact in the field of the sponge holobiont. However I have two major comments that should be considered, followed by minor remarks being more specific.

Major comments

1/ My main comment is about the sample size used, which is the most limiting factor of this study. 3 of the 6 sample groups have 2 replicates or less (Hs norm : n=1 ; Hs anox : n = 2, Es anox : n = 2) which appear to be limiting for proper statistical analyses, especially for differential transcriptomics analyses (Schurch et al., 2016). I believe that despite this small sample size, the informations obtained by the authors are still valuable, and authors did wise choices with the adapted statistical methods used. However I recommend to show more caution in the discussion (as done in the result section) to highlight that some comparisons (especially those involving anoxia for both sponge holobionts) needs to be considered in a study context with a limited number of replicates.

2/ The readability of the article could be improved especially considering the high amount of informations presented in the results section, and followed by the discussion. Here are some suggestions :

- A lot of information is grouped in Figure 1, and the whole figure is actually analyzed all along from L. 387 to 597 and further discussed. As the figure represent an important part of the whole manuscript, it might help the readability to split the figure in two separate ones, for example with one dedicated for the internal comparison with the two sponge species, and another dedicated to the comparisons with the external datasets.

- As many results from the differential analyses were presented, I think that a final figure schematizing all the functional processes discussed for both sponge holobionts (including mitochondria and symbionts) is necessary. I know that it might be a lot of

work, but in overall, I believe that this paper constitute a nice work that deserve a schematic figure to gather all the informations presented. This figure could help the reader to easily get the bigger picture summarizing the main metabolic pathways affected by the hypoxic and anoxic conditions mentioned in the paper, especially considering the HSP, the ATP / glycolysis, and nad2. Moreover, this figure could be presented as a complementary addition to the figure 8 from Schuster et al., (2021).

- Finally, the article suffers from a lack of proper conclusion at the end (after the part of relevance for animal evolution). This conclusion could easily help the reader to get the take home message with the help of the final figure suggested above.

Minor / specific comments

L. 125. to 127 While mentioning the results of Schuster et al., (2021), I would suggest to also indicate that the sampling for the anoxic, hypoxic and normoxic conditions were performed at different sampling times. It helps to consider how this difference of condition goes with seasonal differences (since it's mentioned in the title of the article).

L. 156 to 161. It is still unclear for me to understand exactly when the samples were exactly collected for each oxygen condition, as I cannot open the eps formatted Figure S3 from Schuster et al., (2021) which probably summarizes this information. I think it would be good to also have this information again in this manuscript to avoid to many “round trips” between this paper and Schuster et al., (2021).

L. 292 to 294. (see main comment) Authors pointed the insufficient number of replicates for *H. stellifera* under normoxia (n = 1). I would also suggest to make the same remark for the anoxic condition for both sponge species (n = 2). Does this low number of replicates justify the choice of having a FDR adjusted p-val threshold of 0.1 ?

L. 341 to 350 : Authors mention the methods associated to the phylogenetic positions of nitroreductase proteins. If I am not missing anything, perhaps it would be wise to shortly describe what are the aims / hypothesis associated to this specific part, in the introduction.

Here are below some of my suggestions/questions that could be considered by the authors to enrich their discussion :

L. 758 to 761. Does the oxidative stress induced by lead and zinc concentrations (or other trace metals) could be connected to an inhibition of the water pumping and consequently to an anoxic state, explaining also the upregulation of HSPs ?

L770 to 772. DNA damages are generally linked to an oxidative stress. Hypoxia and anoxia are known to induce such oxidative stress in other models which might affect the integrity of the DNA structure. An upregulation of the DNA repair functions might be explained by the promoted ROS induced by the low oxygen levels (?)

L817 to 818. This might be explained by the following reasons mentionned above : the important DNA damages that could be caused by the oxidative stress during hypoxia could disturb the DNA replication