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Biogeosciences
Discussions

Interactive comment on "Differential gene expression in the mussel *Bathymodiolus azoricus* from the Menez Gwen and Lucky Strike deep-sea hydrothermal vent sites" *by* R. Bettencourt et al.

Anonymous Referee #1

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This study has serious experimental and conceptual flaws. Essential controls are missing and the samples and methods used were inappropriate to answer the questions posed. The manuscript is in many places unintelligible due to unclear writing (particularly in the Abstract) and the Authors rarely attempt to interpret their results.

Experimental concerns:

FISH for bacteria was apparently done with probes called 8F and 1492. These are PCR primers not tested for FISH. The correct general probes to use for all bacteria are EUBI-III (Amann et al. 1990, Daims et al. 1999). Furthermore, if these probes actually worked, why should the signal from these probes only overlap with the signal from

the methanotroph probe (as stated on page 2022, line 6)? They should also target the thiotrophs. Also to the FISH experiments – why was no NON338 (Wallner et al. 1993) negative control included? The images shown for methanotrophs in Lucky Strike mussels in Figure 2 look like natural autofluorescence from the animal tissue, perhaps due to the presence of lipid droplets. This could explain the apparently 'different morphology' the Authors saw for the methanotrophs in Menez Gwen mussels compared to Lucky Strike mussels. The signals for the thiotrophs do not look real, but a NON338 hybridization is essential to assess what the 'real' signal is.

In the materials and methods, the Authors describe using FISH probes for detection of methanotrophs and thiotrophs, but do not cite the original reference (Duperron et al. 2006). In fact, the Authors make the mistake of claiming that these probes target genes for methanotrophy and thiotrophy (in materials and methods text, in the figure legend, and in the discussion). This is incorrect. These probes target the 16S rRNA of the methanotrophic and thiotrophic symbionts.

The cDNA library used for qPCR of bacterial genes was inappropriate as this was subjected to poly-A selection, a treatment designed to remove ribosomal RNA, but which also removes bacterial mRNA. For this reason, the V6 sequencing experiment also has little value, as the PCR products for sequencing were amplified from the same cDNA library.

In the results section, the Authors mention experiments demonstrating upregulation of the immune genes (I assume in Bathymodiolus mussels?) after challenge with Vibrio parahaemolyticus. Why are these results not shown? These are important results that would help to convince the reader of the relevance of the immune genes chosen.

Conceptual concerns:

The Authors found 'no clear overall difference' in the expression of immune genes depending on sampling site. I concur with the Authors that this is not surprising. Why should the expression of immune genes differ depending on sampling site due to dif-

ferences in environmental parameters such as depth or methane and sulfide concentration? I would expect these to change in response to exposure to parasitic bacteria, for example. These mussels are known to have an intranuclear parasite (Zielinski et al. 2009), but factors such as parasite load were not investigated here.

My major conceptual concern in this manuscript is described by the Authors in the final paragraph. They are attempting to compare gene expression in mussels sampled from two different sites and attribute the slight differences they did find to the different geochemical conditions at these two sites. However – since one site is found almost 1000 km deeper than the other, these differences could just as likely reflect the greater stress of decompression at the deeper site. I disagree with the Authors that the absence of appropriate methods for preservation of samples at the sea floor make the methods used here 'adequate for comparative gene expression studies' (page 2028 line 13). The lack of an appropriate method does not justify or validate an inappropriate method.

The second paragraph of the discussion is simply wrong because the Authors did not understand what their FISH probes target (see above).

The Authors do not understand the terms 'methanotroph' and 'methylotroph'. Methylotrophs are microbes that use C1 compounds as a carbon and energy source. Methanotrophs are a subset of methylotrophs that can use methane as a carbon and energy source. The methanol dehydrogenase gene is used by all methylotrophs, including methanotrophs. The expression of this gene therefore provides no evidence that these bacteria are not methanotrophs, as the Authors claim. Also see page 2023 line 17: methylotrophic bacteria that are not methanotrophs do not have genes encoding methane monooxygenases. RuBisCO is not a 'methylotrophy-related gene'. It is the key gene for CO2 fixation by the Calvin-Benson-Bassham cycle. Please see a basic microbiology textbook such as Brock Biology of Microorganisms for more information on these topics.

General concerns about manuscript structure

Results should be explained comprehensively in the Results section. Page 2024 in the Discussion actually belongs in the Results section. The only discussion-like sentence on this page is 'The explanation for this apparent divergence remains a biological challenge that cannot be fully comprehended under the scope of this study'. What was this study for? This style is repeated throughout the Discussion. The Authors describe their results, present a list of facts about either environmental conditions at vents or the roles of immune genes in other animals, but do not attempt to interpret their own results.

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