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Biogeosciences Discussions

Interactive comment on "Influence of chemosynthetic substrates availability on symbiont densities, carbon assimilation and transfer in the dual symbiotic vent mussel *Bathymodiolus azoricus*" *by* V. Riou et al.

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The experimental setup for the various enrichment experiments, including control experiments and wild specimens, used over 100 mussels from the cage recovered in May. Considering that we had to share the cage content with other researchers for different studies, we were already privileged to have access to $\sim$1/4th of the mussels retrieved. We chose to limit our experiments to one concentration level for methane or sulphide, that reproduced conditions observed in situ, to be able to make quantitative interpretations on 14 specimens per enrichment condition. This level of replication is barely reached in published studies of this genus on hydrothermal mussels. The high

standard deviations in incorporation rates are probably due to differential stress causing the fact that some individuals lose their symbionts faster than others, even when held under the same conditions.

No quantitative interpretation was done on the specimens from January. We chose to show the data on tracer distribution in the tissues for a single specimen of each labeling experiment with and without sulphide (Fig. 2), as the exact same trend had been observed in the other specimens analysed. Results from the two other replicates could be included if needed. Data provided for the incorporation of ^{13}C in PLFA can be considered low (n=3), but the standard deviation between specimens was extremely low for the controls (no ^{13}C or no H_2S), indicating that we can be quite confident in these results (control mussels being in exactly the same physical conditions as the mussels incorporating the tracer). The ^{13}C content of other lipid classes were presented for a single specimen, as an example, but the same trend (very low incorporation in apolar lipids fatty acids, ^{13}C appearing first in polar, then neutral lipids fatty acids) had been observed in all analyzed mussels from earlier optimization experiments. We could eventually mention the latter as $<<$data not shown$>>$.

We obviously agree that carboxylation reactions may occur as part of the normal mussel metabolism (i.e., unrelated to chemo-autotrophy), and that such carboxylation reactions might be responsible for part of the observed ^{13}C assimilation. This is unavoidable, and would be a valid remark for all ^{13}C or ^{14}C tracer experiments in such symbioses. However, a number of arguments and patterns in our data can be raised to show that carboxylation reactions should represent only a negligible contribution to the observed ^{13}C assimilation:

1/ Control experiment (i.e. ^{13}C addition in absence of H_2S) indicates a very low ^{13}C uptake (uptake after 15 days in the absence of sulphide is comparable to only 1 day in the presence of sulphide). As carboxylation reactions are similarly expected to occur here, this is a first indication that they represent only a small proportion of the ^{13}C incorporated in the presence of sulphide.

2/ Regarding the hypothesis that sulphide addition may lead to hypoxic conditions and

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thus increase carboxylation reactions, we found no significant differences in O_2 concentrations between experiments with and without sulphide. For January experiments, oxygen saturation stayed between 58 and 96%, with an average saturation of 84% in both (H_2S + ^{13}C) and (^{13}C - H_2S) experiments. May H_2S + ^{13}C experiment was performed at an average saturation of 48% (36 to 72%). Valve closing behavior was not checked, but our monitoring indicated that the seawater used had reasonable levels of oxygen that should not lead to hypoxia.

3/ The clear and consistent pattern in ^{13}C labeling between different tissues (gill $>>$ rest $>$ muscle $>$ mantle) is an indication for the role of symbionts in ^{13}C assimilation. If carboxylation reactions were the main pathway for ^{13}C uptake, we would not expect this pattern to be this pronounced.

4/ Further and direct evidence for the role of symbionts in ^{13}C assimilation is provided by ^{13}C data on individual fatty acids. The specimens that were not supplemented with sulphide did not incorporate significant amounts of ^{13}C in their fatty acids. Plus, an ongoing study indicates that the fatty acids incorporating ^{13}C from methane and labeled bicarbonate in the presence of sulfide (from May specimens gill total lipid extracts) were C14:0 and C16:1 and to a much lesser extent C16:0. This is an indication that mostly the bacteria incorporated the label. $\delta^{13}C$ of C14:0 and C16:1 reached 200 to 2000‰, while most of the other fatty acids had typical background values around -35‰. These values being preliminary, we had chosen not to include them in the manuscript. However, as it seems to help prove the point, we could think about mentioning them.

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