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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 point raised concerning the single specimens shown in figure 2 has been addressed in the reply to Horst Felbeck's comments. Triplicates will be shown in a revised version of the manuscript and the text will be modified. Regarding the lipid fractions, they were isolated from gill tissues only. For the other tissues, only bulk tissue ^{13}C content was analyzed. We agree with the referee that the raised hypothesis of earlier access to symbiont metabolites by the tissues connected to the gills might go a bit too far, and this sentence will be suppressed in the revised version. However, as we mentioned in the discussion, the fact that the <<rest>> of the tissues, which

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contains the digestive tract, is more labeled than mantle or muscle tissues can be due to differential tissue turn-over or indicates that the bacteria are indeed released to the exterior and channeled to the digestive tract. Both symbiont intracellular lysosomal digestion and release into the digestive system might occur at the same time.

The results obtained after incubating mussels with enriched methanol were indeed unexpected. The specimens incubated for 1, 4 and 20 days display identical levels of ^{13}C incorporation in the gill, and label is detected in the muscle tissue of only a single specimen out of 10 after 20 days. Various hypotheses such as a progressive decrease in MOB abundance over time, differential symbiont initial abundances between specimens or unspecific labeling could explain this pattern. We thus preferred not to make hazardous interpretations. Nevertheless, as these are the results obtained, we think that it is appropriate to show them.

Regarding symbiont abundance patterns, the areas occupied by the bacteria were mostly used to evaluate a marked decrease (by a factor $>$ 4) in symbiont abundance between fresh and acclimatized specimens. We think it is meaningful because it correlates with a change in specimen's gill general aspect, and with a drop in gill index. As the reviewer accurately points out, the method employed can only provide $<$rough estimates$>$ of abundance patterns (as we emphasized in the legend from Table 1). Our aim was not to quantify with precision the amount of symbionts but rather to observe population densities in mussel specimens showing the highest ^{13}C incorporation in $<<$mono-diet$>>$ tracer experiments (providing carbon and energy sources either to one or the other symbiont). We thus analysed gill sections from the specimens displaying the highest ^{13}C incorporation from methane after 5 days incubation or from enriched bicarbonate after 4 days in the presence of sulphide. Reviewer remarks that inter individual variability can be high based on his unpublished results. He certainly is right, and because specimens used in the different treatments were distinct, we reformulated the results part, in order to avoid the use of words linked to dynamical aspects (such as $<$increased$>$ or $<$decreased$>$). Despite the low number of replicates, the fact that differences in relative symbiont areas between

BGD

5, S1278–S1281, 2008

treatments fit with what could reasonably be expected, we feel confident about our results (for example, SOB tend to dominate after treatment with H_2S). Of course the analysis of more specimens would have been interesting to strengthen our conclusions with regards to variations in symbiont total and relative abundances. We thus were relatively careful in the discussion (<<Although data must be taken with caution as only one individual per experimental condition was analyzed, the fact that observations qualitatively agree with expected results is a strong indication that our set-up is appropriate for the enrichment of each endosymbiont.>>) and discussed about trends observed and not absolute values.
According to the reviewer's comments, we also modified a bit the discussion, and removed the part about why MOB would resist better than SOB to the absence of their donor, this part being weakly supported by our data and thus mostly speculative. We think the main conclusions are however reasonable given the data provided.

Technical comments will be taken into account in the revised version to modify the material and methods section, but we can already clarify the sampling procedure:
- 3 individuals per condition (experiment with ^{13}C -bicarbonate alone, with sulphide alone or with both) were used in January, while 10 wild specimens were dissected. January mussels were analyzed for gill index (GI) and fatty acid isotopic composition, and for bulk tissue ^{13}C content.
- In May, 10 wild specimens were dissected, their GIs were used to compare with mussels from the tracer experiments of the same month of collection (we admit that mentioning that these mussels were dissected into gill, mantle, muscle and visceral mass was not useful for the present purposes). 1 wild specimen was analyzed by FISH as an example of initial symbiotic population.
- Around 100 mussels were maintained in LabHorta with sulphide and unlabelled bicarbonate and methane before starting various tracer and control experiments, 58 of which were used in this study. 1 specimen was dissected as an example of $t = 0$ for tracer experiments and analyzed by FISH. 1 of the mussels that remained 4 additional

BGD

5, S1278–S1281, 2008

days in maintenance conditions was sampled for FISH. At the beginning of the tracer experiments, 14 specimens per condition were transferred to the different tanks (containing labeled methane, labeled bicarbonate with sulphide, labeled methanol, or no added chemical = control). 1 individual of each enrichment tank was dissected after 1 day, 3 specimens after 4 days (for sulphide, methanol and control experiments) or 5 days (for the experiment with methane) and 10 specimens after 20 days (for sulphide, methanol and control experiments) or 15 days (for the experiment with methane).
We also agree that mentioning that mantle and visceral mass were also dissected from mussels from the tracer experiments was of no use, as we do not refer to these tissues in the results. All May experiment mussels were analyzed for GI, gill and muscle bulk stable isotopic compositions. FISH analyses were performed on 1 mussel from the sulphide and 1 from the methane experiments, each showing highest ^{13}C incorporation, and on 1 random mussel from the control experiment.

One sentence was modified to make clear that ImageJ was employed for the processing and analysis of obtained FISH whole images. All the filaments of each picture were analysed (one image per specimen, around 20 filaments per image) and bacterial area estimations were normalised by the total filament length present on each image.
Caption of figure 2 will be corrected for the misleading error attributing mussel A to the control without sulphide.
On Fig. 4, only the specimens incorporating detectable amounts of ^{13}C are plotted. After 5 days in $^{13}CH_4$, only 1 of the 3 sampled specimens had incorporated detectable amounts of ^{13}C, this is why only one dot is visible. The latter is well separated from the 2 methanol specimens that incorporated detectable amounts of ^{13}C, as the methanol specimen comes later on the time scale. But we agree that dots and axes descriptions are definitely too small in figure 4 and this will also be changed.

Interactive comment on Biogeosciences Discuss., 5, 2279, 2008.