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Biogeosciences Discussions

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Interactive Comment

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.

C. Borowski (Referee)

cborowsk@mpi-bremen.de

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The manuscript by Riou et al. investigates carbon fixation and symbiont abundances in the dual symbiosis of the hydrothermal vent mussel Bathymodiolus azoricus in response to experimental manipulation of supply with chemosynthetic substrates H2S and CH4. Mussels were exposed over several time intervals to various combinations of 13C labelled substrates and C incorporation rates were calculated from isotopic signatures in mussel tissues and in lipid fractions isolated from separate tissues. Quantitative analyses of symbiont densities in gill tissues using FISH were used to support the

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stable isotopic data. The experiments were performed in aquaria at Lab-Horta under atmospheric pressure, which was possible because Menez Gwen mussels survive and recover from an apparently moderate decompression impact during the retrieval from only 850 m water depth, and previous investigations have demonstrated that physiological experiments give reliable results. This is fascinating and I very much appreciate the experimental approach, however, I have strong concerns towards the presentation of some of the data and the way how conclusions are drawn.

Chemosynthetic assimilation of carbon by methanotrophic endosymbionts of Bathymodiolus species is undisputed and the worth of in vivo experiments as they were performed here is that they can bring up realistic quantitative data. However, data measured in single individuals as representatives for entire treatments but without estimations of the variances within treatments do not allow estimating the significance of the results. Thus, shifts of d13C signatures in tissues of only one treatment individual (versus control measured in considerable sample size: n = 10) remain incidental (Fig. 2). Mentioning that other data may exist (p. 2293 line 8) does not help here. These must be shown to support the conclusions. P 2289 line 9: How can lower increase in d13C be significant, when only one individual was measured?

The applied method of calculating 2D-areal distribution patterns of the bacteria in gill tissue cross sections may be suitable for a rough estimation of abundance patterns of symbionts in treatments. However, natural variability between individuals must be considered and comparisons on the basis of only single individuals per treatment is impossible. Our own preliminary data on symbiont biovolume in the dual symbiotic B. puteoserpentis from the Logatchev hydrothermal vent field (measured by 3D FISH as μm^3 biolvolume / bacteriocyte) vary up to factor 2 between individuals. This indicates considerably higher variability than the values for symbiont biovolume per bacteriocyte in B. azoricus published by Halary et al (2008), however, no information exists on inter-individual variability in this host species. The variation of the here reported areas (1.7 - 5.1 μm^2 surface / μm filament) covered by symbionts in single specimens of different

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treatments matches the range of natural variability observed among B. puteoserpentis individuals and therefore should not be used for an interpretation of treatment effects.

Mid-p. 2293: Why should tissues next to gills benefit earlier from symbiotic carbon assimilation than other tissues? Metabolic products should be distributed in the host body mainly with the stream of the haemolymph rather than by pure diffusion. Conventional feeding on symiotic bacteria implies that the gills must release symbionts to the exterior. Is there any evidence for such behaviour in B. azoricus? On the other hand, direct response of gill tissue to isotopic treatments is obvious because the gills host the primary producing bacteria, while isotopic equilibrium in other host tissues can only be reached after longer time of metabolization.

In contrast to the authors, I am quite convinced that there is incorporation of 13C after incubation with methanol. Although Fig. 4 is very small it suggests some 7 and 5-20 μmol C / g dry gill after 4 and 20 days, respectively, and 5 μmol C / g dry muscle after 20 days, and I suspect that consequent statistical treatment of the data might have revealed significant increase of net incorporated C over the entire 20 d period. These values at least fall within the lower range of 13C incorportion in the presence of methane. Methanotrophs are per definition methylotrophs that have monooxigenases with which they oxidize methane to methanol before this (here) intermediate enters the ribulose monophosphate (type I) or serine (type II) pathways for assimilation of C1 compounds, and I do not see a reason why methanotrophs should not be able to use methanol as a substrate. Exposure to methanol therefore must be seen as a treatment rather than a control experiment, and the discussion should rather focus on the question why methanol utilization is so much less efficient compared to the utilization of methane (for example: are bacteriocyte membranes less permeable for methanol than for methane?). However, this opens a new door to questions otherwise not addressed, and it should be considered whether the methanol issue should remain in the manuscript.

Technical comments

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Apart from the above comments, I suggest to improve the comprehensibility of the writing by better structuring.

Some examples:

Instead of misleading the reader concerning the amount of expected data by giving numbers of animals recovered with the cages the authors should explain in the methods how many individuals were used for which analyses (see also comment by Horst Felbeck). The authors should make transparent if GI, FA and FISH was analysed from the same specimens. Why were mantle and visceral mass dissected from 20 d incubation specimens when FAs and d13C signatures were apparently not analyzed?

Some methods descriptions are very short and would benefit from better explanations. E.g. 2D FISH counting: What does ImageJ analyse? How many bacteriocytes or filaments were analysed?

Individual mussel A is only introduced late in the discussion and while reading results its importance must be riddled from the figures. Attribution to the wrong treatment in the caption of figure 2 makes it even more complicated.

Fig. 4 plays a central role in the manuscript but its presentation is poor. The small size makes it for example impossible to discern how many measurements went into 4/5 day methanol and CH4 treatment data.

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

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