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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 double symbiosis of methane and sulfur oxidizing bacteria with the mussel Bathymodiolus azoricus is one of the most exciting symbioses found recently. The ability to use alternative energy sources, methane or sulfide, is enhanced by the observed possibility to adapt the composition of the population of symbionts to the availability of the respective chemicals. When methane is present in the surrounding seawater, the methane oxidizing symbionts dominate while the sulfide oxidizing symbionts are more common with sulfide in the water. Some changes of the physiology of the mussels in

response to these events have been published previously. The manuscript concentrates on the changes of the symbiont population and the physiology of the symbiotic bacteria, especially the carbon fixation rates from different carbon sources. The authors have access to a symbiotic system that allows them to perform experiments at atmospheric pressure, an invaluable benefit when many of these deep-sea symbioses have to be maintained in pressure aquaria. It is therefore a little disappointing that the number of specimens in the experiments (“n”) are as low as presented, especially since so much quantitative interpretation of the results are presented. The authors should mention why in the presence of 400 collected specimens only few were used. The determination of carbon dioxide fixation rates based on tracer studies is very ambiguous and has to be interpreted very carefully and cautiously. There are too many pitfalls that can change the numbers. One of the most significant problems is the apparent “fixation” of CO2 when in fact no net fixation occurs. This phenomenon can easily be observed when a carboxylation step is involved in the intermediary metabolism. The labeled carbon incorporated during this carboxylation step is subsequently diluted in the pool of non-labeled metabolites within the cell following the carboxylation step. When these metabolites are then decarboxylated again later in the intermediary metabolism, the molecule decarboxylated does not have to be the one carrying the label but can be any of the present ones, including the non-labeled ones. Thus, a fixation of label can be measured while no “net fixation” has occurred. One of the most significant carboxylation steps in mytilid bivalves occurs when the normal glycolytic step from phosphoenolpyruvate to pyruvate is switched to the carboxylation step from phosphoenolpyruvate to oxaloacetate when hypoxic conditions are encountered. Basically the entire (!) energy metabolism of mytilids using glycogen as substrate is channeled through this step during anaerobic conditions. The manuscript demonstrates that in the presence of sulfide, “fixation” rates of CO2 increase. If the mussels should become hypoxic during the experiment, e.g. due to chemical reactions between sulfide and oxygen or closing of their valves after entrapping label in the valve cavity, the observed results could

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easily be explained by this carboxylation reaction. Individual valve closing behavior of various experimental specimens could also explain the variations of the incorporation results. In addition, the hypoxic metabolic pathway involving the carboxylation step mentioned above leads directly to immediate metabolic precursors of fatty acids possibly shunting “fixed” label into the measured pool of fatty acids and lipids. The authors mention that the oxygen concentration during the experiments were controlled but do not provide the data. These should definitely be given since they are crucial for the interpretation of the results. If at any time the oxygen concentrations are exceedingly low (unfortunately no solid number can be given as it varies for different species…) the data should be discussed accordingly. It also should be assessed how frequently and for how long time periods the mussels closed their valves after introducing the labeled CO2.

Technical comments: I am very pleased about the English used in this manuscript. It is a vast improvement over some other manuscripts. There are still a few typos and grammatical errors listed in the following: The authors should replace the verb “to evidence” with something like “observe”, “demonstrate”, “prove”, or similar throughout the manuscript. Line 9 page 2282: should be “proteobacteria” Line 12 page 2283: was that seawater buffered? If yes with what? Line 17 page 2285: see comments above Line 27 page 2292: How should ATP get into the bacteria? Line 16 page 2293: should be…. Grown in the mussel cell, could have been released into the food…”

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

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