Geophysical Research Abstracts,
Vol. 10, EGU2008-A-09210, 2008
SRef-ID: 1607-7962/gra/EGU2008-A-09210
EGU General Assembly 2008

3D FISH for the quantification of methane- and sulphur-oxidising endosymbionts in bacteriocytes of the hydrothermal vent mussel *Bathymodiolus azoricus*

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Dual endosymbioses involving methane- and sulphur-oxidising bacteria occur in the gills of several species of mussels from deep-sea hydrothermal vents and cold seeps. Variations of total and relative abundances of symbionts depending on local environmental parameters are not yet understood, due to a lack of reliable quantification of bacteria in the host tissue. Here we report the first attempt to quantify volumes occupied by each type of symbiont in bacteriocyte sections from a vent mussel, *Bathymodiolus azoricus*, using fluorescence *in situ* hybridization (FISH) coupled to 3D microscopy and image analysis carried out by a dedicated software which we developped. Bacteriocytes from mussels recovered at different vent sites displayed significantly different abundances of bacteria. Specimens kept in aquaria at atmospheric pressure and exposed to an artificial pulse of sulphur displayed an increase in absolute and relative abundance of sulphur-oxidisers within their bacteriocytes. Distributions of all measured parameters fitted normal distributions, indicating that bacteriocytes from a specimen tend to display similar behaviours. This study shows that symbiont

volume quantification is tractable using 3D FISH and confirms the impact of local environmental parameters on symbiont abundances.