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Chemotrophic carbon fixation and transfer in the dual endosymbiotic mytilid *Bathymodiolus azoricus* from the Mid-Atlantic Ridge

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The coexistence of two distinct bacterial symbionts within a single cell of a multicellular eukaryote was demonstrated for the first time ever in the gills of the deep sea mytilids (*Bathymodiolus* spp.) (Cavanaugh *et al.*, 1987; Cavanaugh *et al.*, 1992; Fisher *et al.*, 1993; Robinson *et al.*, 1998). Such mytilids are present at hydrothermal vents of the Mid-Atlantic Ridge (MAR). *B. puteoserpentis* and *B. azoricus*, the two mussel species present at MAR vent sites, display the same general characteristics, with two phylogenetically distinct (Distel *et al.*, 1995) morphotypes of Gram (-) endosymbionts in gill bacteriocytes, associated with the immuno-detection of enzymes specific for sulphide and methane oxidising metabolisms (Fiala-Médioni *et al.*, 2002). Duperron *et al.* (2006) observed that vent chemistry would affect the relative abundance of thiotrophs and methanotrophs. The volume occupied by each type of symbiont present in a bacteriocyte, inferred from a new 3D FISH technique (Halary *et al.*, 2007), was found to be variable between *B. azoricus* specimens from different sites. In this same study, we also demonstrated that the presence of sulphur compounds promotes the rapid growth of sulphur-oxidisers, even at atmospheric pressure. We recently performed, on specimens from Menez Gwen, stable isotope tracer experiments

with ^{13}C-enriched bicarbonate, methane or methanol, in a controlled laboratory environment (LabHorta facility in the Azores) followed by EA-IRMS analysis of the mussel gill and muscle tissues. We provide the first evidence for physiological activity of the symbionts in live specimens of *B. azoricus* based on the assimilation of carbon from the oxidation of inorganic and C1-substrates by the endosymbionts (at atmospheric pressure) and its translocation to symbiont-free mussel tissues. The dynamics in the relative proportions of symbionts in each enrichment condition are monitored by 3D-FISH and the rate of carbon incorporation by each symbiont is quantified and discussed.