



Combining approaches to understand the ecology of symbiotic invertebrates from chemosynthetic ecosystems

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Symbiotic associations between chemosynthetic bacteria and invertebrates are among the major features characterizing deep-sea chemosynthetic environments. Mussels of the genus *Bathymodiolus* (Bivalvia: Mytilidae) are typical invertebrates dominating several cold seeps and hydrothermal vents worldwide. Some species are associated with both chemoautotrophic and methanotrophic bacteria, making them a good model for studying the ecology of symbioses. However, despite intensive efforts, interactions between these symbiotic systems and their environment are still poorly understood, due to technical difficulties in performing *in situ* experiments and measurements.

Three examples combining different tools in an integrative way to investigate the ecology of dual-symbiotic mussels will be presented. The first two examples are attempts to link vent and seep fluid composition to the relative abundances of chemoautotrophic vs methanotrophic symbionts. Mussels from several Mid-Atlantic Ridge hydrothermal vent sites, with differing end-member fluid compositions, were shown to display different relative abundances of symbionts in their gills, based on visual inspection of gill filament sections hybridised to symbiont-specific fluorescent probes (FISH). In the second example mussels collected, in parallel to water samples, on three aggregates on a methane seep site of the Gabon margin showed no differences in relative abundances of symbionts, despite an up to 50-fold variation in methane concentration between aggregates. In the last example, bacterial abundances in mussels from dif-

ferent sites in the Gulf of Mexico were quantified by rRNA slot-blot hybridisation, controlled with FISH, and stable isotope analyses determined, with all 3 indices measured in parallel samples from the same individuals. Both FISH and rRNA slot-blot hybridization confirmed that bacterial abundances differed between sites. Heavier stable carbon isotope signatures indicated a lesser importance of methanotrophic bacteria in the nutrition of specimens with lower bacterial abundances. These results suggest that symbiont abundances and host nutrition are affected by site-specific differences.

Much still has to be done to understand the ecology of symbiotic organisms. Combining different quantitative approaches like molecular biology, stable isotope analysis and *in situ* chemistry at scales relevant to the organisms will be a key point to be considered for future studies, as we hope to demonstrate in this presentation.

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