Nitrogen assimilation pathways in two dominant demosponge species inhabiting the North East Atlantic Cold-Water Coral Reefs

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Cold Water Reefs (CWRs) are a spectacular type of deep-sea ecosystems with high ecological (e.g. "hot spots" of biodiversity) and financial importance (e.g. nursery grounds of commercial fish) (Roberts et al. 2009; Henry et al. 2013). In the North East Atlantic Ocean, CWRs are present off Ireland, Rockall Bank (NE Atlantic) and on the Norwegian shelf where the hydrographic conditions promote food supply to the reef organisms (Duineveld et al. 2007, 2012; White et al. 2012). The increased supply of food particles towards the reef is thought to be one reason for the ecosystem's characterisation as a "hot spot" of biomass, nutrient cycling and biodiversity (van Oevelen et al. 2009).

In the CWRs of the North East Atlantic Ocean, sponges have a widespread distribution (van Soest et al. 2007; Roberts et al. 2009). Despite this fact, our knowledge on the biology of reef sponges -and especially in their ecophysiology and trophic ecology- is very limited (van Duyl et al. 2008). This scarcity of information hinders our understanding of the function of sponges in cold-water coral reef food webs, energy supply and nutrient cycling.

During the "Changing Oceans" expedition (James Cook 073) in May/June 2012 we investigated the role of sponges in the cold water coral reef's food web through experimentation with particulate and dissolved organic and inorganic substrates labelled with carbon (^{13}C) and nitrogen (^{15}N) stable isotopes. The substrates that were used were glucose (^{13}C), NH_4Cl (^{15}N), the diatom *Thalassiosira rotula* (^{13}C, ^{15}N) and bacteria (^{13}C, ^{15}N). The species that were used in the experiments were the demosponges *Spongosorites coralliophaga* (Stephens, 1915) (collected from Mingulay 1 at Mingulay Reef Complex and Logachev Mounds at the Rockall Bank) and *Mycale (Mycale) lingua* (Bowerbank, 1866) (collected from Pisces 9 Site). *S. coralliophaga* is a massive sponge and has high abundance in Mingulay area (Vad, 2013), characteristics that can be regarded as a promising indication around the role of this sponge species in the functionality of the Mingulay Reef ecosystem.

Preliminary results from our experiments have shown differences between the used substrates, in terms of utilisation pathways. Taking into account these observations, as well as previous findings highlighting the complex nitrogen cycling within the "sponge-microorganisms" consortium (Hoffmann et al. 2009; Maldonado et al. 2012), we wanted to investigate further the nitrogen-uptake mechanisms in these deep-sea demosponges and in particular to study and quantify the relative contribution of sponge cells and symbiotic bacteria. Our approach was based on novel techniques using ^{15}N isotopic analysis in Hydrolysable Amino Acids (HAAs), incorporating D-Ala which is bacteria-specific amino acid (Veuger et al. 2005, 2007).

Sponge samples were sub-sampled, lyophilised (−60◦C; −0.0001 mbar; 72 hrs) and grinded using TissueLyser II (Qiagen). Following steps involved hydrolysis, derivatization and isotopic amino acid analysis through gas chromatography-combustion-isotope ratio mass spectrometry (GC-c-IRMS) (Veuger et al. 2005, 2007). Analysis of samples was done in collaboration with Dr Bart Veuger in the Netherlands Institute of Ecology (NIOO-KNAW). The Deep-Sea Forum Grant (470 GBP) was used for the chemical analyses mentioned above. The data set produced is going to be statistically analysed and the produced manuscript will be submitted for publication in a peer-reviewed journal in 2015.

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