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The Use of Hypercalcified Sponges as Proxy Indicators of Climate Change: Biological Investigations

L. Berry (1), V. Sivel (2), H. Zandbergen (2), Ph. Willenz (1) and the CALMARS group.

(1) Department of Invertebrates, Royal Belgian Institute of Natural Sciences, Brussels, B1000, Belgium

(2) Laboratory of Materials Science, Delft University of Technology, Rotterdamsweg 137, 2628 AL Delft, The Netherlands

Corresponding author: Lorraine Berry. E-mail address: l.s.berry@tnw.tudelft.nl

The massive basal skeleton of hypercalcified sponges has stimulated much interest in recent years due to their potential to provide information on past climate. One species in particular, *Ceratoporella nicholsoni*, is known to be a proxy indicator of past climate [1,2]. A lead profile along the skeleton growth axis of this species has been shown to increase and decrease relative to atmospheric pollution [1]. Although accepted as a proxy indicator, little is known about the calcification processes in these sponges, or about the incorporation of elements into the skeleton during calcification. In addition, the relationship between elements in seawater and in the skeleton has not yet been determined.

We investigated the uptake route of elements into the tissues of *Petrobiona massiliana*, an easily accessible Mediterranean hypercalcified sponge. This species lives in shallow submarine caves and secretes a massive calcitic basal skeleton. The element uptake route may infer whether the relative concentrations of elements incorporated into the skeleton could be altered by physiological factors. For example, active transport and membrane selectivity.

Sponges were incubated *in situ* with visible particulates (ferritin; c.12.2 nm diameter) and bacteria (grown in strontium-rich media) to provide a visual uptake route of particles into the sponge and to investigate the potential uptake of elements bound or incorporated into organic matter. Incubations were also performed with dissolved and precipitated (particulate) ions (Sr^{2+} and Ba^{2+}). Transmission Electron Microscopy (TEM) observations showed uptake and accumulation of particles inside endocytic vesicles of specific cell types. Bacteria marked with strontium were also accumulated within phagosomes of the same cells. Incubations with the dissolved elements (up to 10 mM) did not allow the location of elements to be determined within the sponge tissue. However, supersaturated incubations (providing particulate ions) allowed element hotspots to be discovered using Scanning Electron Microscopy (SEM) + Energy Dispersive X-ray analysis (EDX). Focused Ion Beam-cut sections from the element hotspots enabled positive analysis using the Scanning TEM + EDX, but the exact cellular locations are still to be determined at the ultrastructural level.

This work is a necessary step in the investigations of element uptake and biomineralisation processes in hypercalcified sponges, and towards the biological validation of these sponges as reliable proxy indicators of climate change.

[1] Lazareth C.E., Willenz Ph., Navez J., Keppens E., Dehairs F., and André L. (2000). Sclerosponges as a new potential recorder of environmental changes: Lead in *Ceratoporella nicholsoni*. *Geology*, 28 (6): 515-518.

[2] Rosenheim B.E., Swart P.K., Thorrold S.R., Willenz Ph., Berry L. and Latkoczy C. (2004). High-resolution Sr/Ca records in sclerosponges calibrated to temperature *in situ*. *Geology*, **32** (2): 145-148.