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Insights into the Skeletal Growth Pattern of the Hypercalcified Sponge *Petrobiona massiliana*

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Interest in calcifying marine organisms, including hypercalcified sponges, has grown over the last 20 years because of their potential to provide environmental information relating to past climate [1,2]. As a result, growth rates and knowledge of skeletal development have become increasingly important. Hypercalcified sponges secrete a massive basal skeleton that can be analysed along the growth axis, providing environmental information accumulated during the lifetime of the specimen. *Petrobiona massiliana*, a hypercalcified sponge, secretes a massive calcitic basal skeleton. Although small, this species could potentially provide climatic information at different points in time. Living specimens are found in the Mediterranean, growing in submarine caves [3]. In addition, subfossil skeletons have been found, dating back 1500 years ago, and fossil remains, dating back 30 000 years [4]. However, before any geochemical analyses can be interpreted, the growth rate and development of the skeleton have to be determined.

The growth rate of *Petrobiona massiliana* was investigated using calcein, a fluorochrome that binds permanently at the site of precipitation of calcium carbonate [5]. Whole specimens were glued to plates to allow ease of *in situ* incubation with calcein. Annual growth rates were highly variable. The mean annual growth rate for the specimens allowed to recover after plating was $242.76 \pm 161.42 \ \mu$ m. Measurements taken from the calcein labelling performed at the time of plating were significantly lower (56.66 \pm 23.67 μ m), suggesting that manipulations had affected growth rate. Measurements also varied significantly from one specimen to another, ranging from 111.60 \pm 57.21 μ m to 676.69 \pm 63.80 μ m for those allowed to recover, and from 9.72 \pm 3.37 μ m to 70.17 \pm 40.81 μ m for those incubated with calcein soon after plating. The growth rate of this species falls within the range observed in other hypercalcified sponges [2,5,6].

Scanning Electron Microscopy (SEM) observations of the skeleton showed multidirectional growth of the surface skeletal projections, which made the skeletal growth axis difficult to distinguish during growth measurements. High Resolution SEM was also performed to search for surface structures that may indicate centres of calcification, increasing our understanding of skeletal development and growth in this species, and their use as proxy indicators of climate change.

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