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Visualization of sub-daily skeletal growth patterns in massive *Porites* corals grown in Sr-enriched seawater

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ABSTRACT

We performed high resolution marking experiments using seawater with elevated Sr concentration to investigate the timing and ultrastructure of skeletal deposition by massive *Porites australiensis* corals. Corals were cultured in seawater enriched with Sr during day-time only, night-time only or for one full-day. Cross sections of skeletal material were prepared and the Sr incorporated into the newly deposited skeleton analyzed by electron probe microanalysis. These regions of Sr incorporation were then correlated with skeletal ultrastructure. Massive *Porites* coral skeletons are composed of two types of microstructural elements – the "centers of calcification" and the surrounding fibrous structural region. Within the fibrous structural region, alternative patterns of etch-sensitive growth lines and an etch-resistant fibrous layer were observed. In the full-day samples, high-Sr bands extended across both growth lines and fibrous layers. In day-time samples, high-Sr regions corresponded to the fibrous layer, while in the night-time samples high-Sr regions were associated with an outermost growth line. These distinct growth patterns suggest a daily growth pattern associated with the fibrous region of massive *P. australiensis* corals, where a pair of narrow growth lines and a larger fibrous layer is seen as a daily growth region.

1. Introduction

Hermatypic scleractinian corals are widely distributed from tropical to temperate areas, forming calcium carbonate exoskeletons that act as the foundation of coral reef environments. The complex framework formed by coral reefs has resulted in these systems being one of the most diverse and socio-economically important habitats on Earth. One of the key questions that has remained unanswered for more than a century, has been how do these corals grow and deposit their skeleton, in often very fast time frames, to form this complex reef framework? In the face of climate change and ocean acidification, understanding the mechanism of coral calcification has become an important topic in coral reef science (Hallock, 2005; Watanabe et al., 2007; Anthony et al., 2008; Wei et al., 2009; Cohen and Holcomb, 2009).

Considerable debate exists as to the mechanisms underlying the role of symbiotic algae, known as zooxanthellae, in the calcification process. Traditionally, calcification in zooxanthellate corals has been considered 'light enhanced', with calcification rates observed to be much higher during day time than night time, with this increase attributed to the photosynthetic activities of the zooxanthellae. However, data from azooxanthellate corals indicates that these corals can calcify at comparable rates to zooxanthellate corals (Marshall, 1996; Marshall and Clode, 2004; Tambutté et al., 2007; Maier et al., 2009), fuelling the ongoing debate as to whether calcification in zooxanthellate corals is truly light enhanced. Further, calcium uptake is known to be light sensitive, although the mechanisms behind this are unclear (de Beer et al., 2000; Al-Horani et al., 2003; Marshall and Clode, 2003).

Diurnal patterns of coral biomineralization, such as the cycling of calcification rate and depositional timing of specific ultrastructures, may provide unique opportunities to study the process of coral biomineralization and the potential effects of light upon this. It is well established that coral skeletons are composed of well arranged microstructural elements (e.g. Cuif and Dauphin, 1998; Sto-

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larski, 2003; Nothdurft and Webb, 2007; Janiszewska et al., 2011). It has been accepted for many years that the fundamental ultrastructural units are so called "centers of calcification" surrounded by fibrous skeleton (e.g. Bryan and Hill, 1941; Cohen and McConnaughey, 2003). However, recent advances in microstructural studies suggest that this definition of ultrastructural units is not adequate (Cuif and Dauphin, 1998, 2005a, 2005b; Cuif et al., 2003; Stolarski, 2003; Nothdurft and Webb, 2007; Janiszewska et al., 2011). As our understanding of skeletal ultrastructure and formation further develops, various new terminologies have been introduced, dependent upon the discipline (geo or bio) and on the possible mechanisms of formation. Here we use the traditional terminology of "center of calcification" and fiber because this terminology is, to our knowledge, the most widely recognized and used to date. However it should be noted that recent studies (Cuif and Dauphin, 1998, 2005a,b; Cuif et al., 2003; Stolarski, 2003; Nothdurft and Webb, 2007; Janiszewska et al., 2011) and the current authors do not support idea that the "center of calcification" functions as the name implies.

Microstructural observations of the skeletal surface determined by time-series sampling has revealed that deposition of different microstructural elements may occur at day time and night time in a variety of corals, including *Manicina aereolota* (Barnes, 1972), *Acropora cervicornis* (Gladfelter, 1982, 1983), and *Pocillopora damicornis* (Le Tissier, 1988). Using repeated staining with alizarin red Sandeman (2008) also found that the superimposed lamination of optically denser and lighter bands in fibrous region of *Agaricia agaricites* were formed during the day and night respectively. In contrast, no clear diurnal patterns were observed in *Galaxea fascicularis* (Hidaka, 1991; Clode and Marshall, 2003a). Raz-Bahat et al. (2006) also reported no diurnal pattern of growth in *Stylophora pistillata* using a lateral skeleton preparative assay. Such inconsistent results suggest that calcification patterns and diurnal processes may be highly species specific, although this also implies that daylight and zooxanthellae are not central to driving this process.

There are several methods that have been used to investigate the skeletal growth patterns of corals, each with advantages and limitations. These include the use of skeletal dyes (Isa, 1986; Böhm et al., 2006) and radioactive tracers (Marshall and Wright, 1998; Tambutté et al., 1996; Furla et al., 2000; Ferrier-Pages et al., 2002), time-lapse photography (Barnes and Crossland, 1980), and ion micro-sensors (Al-Horani et al., 2003; Marshall and Clode, 2003). Skeletal dyes are probably the most straightforward method to visualize growth in a given time. Barnes (1970) and Sandeman (2008) successfully identified skeletal deposition at an hourly scale using alizarin staining. However, the major drawback of this chemical staining method is the potential toxicity of the dye, which may cause decreases in calcification rate (e.g. Dodge et al., 1984; Gaetani et al., 2011). Recently, Houlbreque et al. (2009) used the stable non-toxic isotope tracer ^{86}Sr to visualize skeletal deposition in *Porites porites* over 3 days by imaging ^{86}Sr distribution within the skeleton using high resolution ion microprobe analyses (NanoSIMS). However, our ICP data show that ^{86}Sr does not dissolve into seawater when simply added as solid $^{86}SrCO_3$ as reported by Houlbreque (unpublished data), questioning the method of Sr uptake into the tissues and skeleton in that study.

Understanding the mechanisms of coral skeletal deposition and growth, and the factors affecting these, is also fundamental to the field of coral geochemistry, where Sr/Ca, Mg/Ca and O isotopic ratios are intensively used as past seawater temperature proxies (see reviews by Gagan et al., 2000; Lough, 2004; Correge, 2006; Watanabe et al., 2007). Microanalytical studies have indeed revealed that there are significant compositional heterogeneities that cannot be explained by temperature alone (Cohen et al., 2001; Meibom et al., 2004, 2006, 2008; Allison and Finch, 2004, 2007; Shirai et al., 2005, 2008; Gaetani and Cohen, 2006; Holcomb et al., 2009; Allison et al., 2010; Gaetani et al., 2011). Since this heterogeneity is strongly associated with skeletal ultrastructure, the source of the heterogeneity is considered to be of, as yet unknown biological origin.

In some studies, elemental fractionation is thought to be derived from ultrastructural variation, with compositional differences between "centers of calcification" and fibrous regions of massive *Porites* corals attributed to diurnal differences, based upon the assumption that these microstructural elements are formed during night and day respectively (Cohen et al., 2001; Cohen and McConnaughey, 2003; Allison and Finch, 2004, 2007). However, diurnal patterns of skeletal growth regions have not been reliably investigated, thus this assumption is largely unfounded. Massive *Porites* corals are the most commonly used genus for paleoclimate reconstructions, therefore to accurately interpret the mechanisms of microscale elemental fractionation, a detailed understanding of ultrastructural formation is essential.

With these considerations in mind, it is clear that more information is needed in regard to the patterns of skeletal deposition and growth in corals and the biotic and abiotic factors that affect and control these. In this study we present a versatile method to visualize sub-daily growth patterns of massive *Porites australiensis* corals by high temporal resolution marking experiments performed using seawater enriched in Sr concentration. We have correlated this pattern of Sr deposition with skeletal ultrastructure and obtained skeletal deposition patterns over day time and night time.

2. Materials and methods

2.1. Culture experiments

Culture experiments were performed at Shiraho Reef, Ishigaki Island, Japan (Fig. 1). Detailed environmental settings of Shiraho Reef are described in Appendix A. Since this study attempted to investigate coral growth over short time scales, experiments were designed to minimize any environmental or mechanical stress on the corals during the sampling process and experimentation. Cuts were made in massive *P. australiensis* colonies from ~1 m depth at several cm intervals and to a depth of ~5 cm with a saw. These pieces of coral were allowed to recover for two full days, after which the basal part of the coral colony where no living polyps persist, was mechanically broken. The resulting blocks, of which the top surface was covered with living polyps, were subsequently used for the culture experiments. All replicate experimental blocks were sampled from the same colony. Only corals that extended their tentacles out from the calyx were used for experimentation.

The experiment was conducted at the border area between the moat and the inner reef flat of Shiraho Reef. Seawater temperature was measured every hour using a data logger placed within 500 m of the experimental site (Fig. 2). Salinity was measured 2 km north of the experimental site at approximately 16:30 and was found to be 34.5 psu. Solar radiation and tidal data were obtained from the meteorological station located ~15 km south west of Shiraho Reef. Relevant environmental data are summarized in Fig. 2. Photon flux density was roughly estimated by the relationship between the values reported by Nakamura and Nakamori (2009) and the solar radiation data (Photon flux density ($\mu mol\ m^{-2}\ s^{-1}$) = 446× solar radiation ($mJ\ m^{-2}$), $n = 14$, $R^2 = 0.93$). The low tides were observed at 7:30 and 18:30, and the high tides were observed at 13:40 and 1:00.

For incubation, 10 L tightly sealed plastic containers with 84% sunlight transparency were used. Coral samples ($n = 4$–5) were transferred into these containers without air exposure. Solid