The morphology and composition of abiogenic (synthetic) aragonites precipitated experimentally from seawater and the aragonite accreted by scleractinian corals were characterized at the micron and nano scale. The synthetic aragonites precipitated from supersaturated seawater solutions as spherulites, typically 20–100 µm in diameter, with aggregates of sub-micron granular materials occupying their centers and elongate (fibrous) needles radiating out to the edge. Using Sr isotope spikes, the formation of the central granular material was shown to be associated with high fluid pH and saturation state whereas needle growth occurred at lower pH and saturation state. The granular aggregates have significantly higher Mg/Ca and Ba/Ca ratios than the surrounding fibers.

Two types of crystals are identified in the coral skeleton: aggregates of sub-micron granular material and bundles of elongate (fibrous) crystals that radiate out from the aggregates. The granular materials are found in “centers of calcification” and in fine bands that transect the fiber bundles. They have significantly higher Mg/Ca and Ba/Ca ratios than the surrounding fibers.

The observed relationship between seawater saturation state and crystal morphology and composition in the synthetic aragonites was used as a framework to interpret observations of the coral skeleton. We propose that coral skeletal growth can be viewed as a cyclical process driven by changes in the saturation state of the coral’s calcifying fluids. When saturation state is high, granular crystals precipitate at the tips of the existing skeletal elements forming the centers of calcification. As the saturation state decreases, aragonitic fibres grow in bundles that radiate out from the centers of calcification.

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1. INTRODUCTION

Early studies of the ultrastructure of the scleractinian coral skeleton identified two distinct structures: the centers of calcification which appear as dark spots in petrographic thin-section, and the clusters of fibrous crystals which radiate out from the centers (e.g., Ogilvie, 1896; Vaughan and Wells, 1943; Wells, 1956). The centers of calcification and their clusters of fibers are called sclerodermites and are considered to be the basic building blocks of the skeleton (Wells, 1956). Bryan and Hill (1941) noted the striking similarity between spherulitic morphologies observed in a range of mineral systems, and coral sclerodermites. Both exhibit fibrous crystals radiating from a common center. Spherulitic growth in mineral systems is associated with diffusion-controlled growth from highly supersaturated solutions (Keith and Padden, 1963; Chernov, 1984). Such observations led Barnes (1970) and Constantz (1986), among others, to describe the precipitation of aragonite by scleractinian corals as a process analogous to crystal growth from highly supersaturated solutions.
Over the past few decades, a range of imaging and analytical techniques have been employed to examine coral skeletons. SEM imaging of materials occupying the centers of calcification showed that these are morphologically distinct from the surrounding fibers. The materials at the centers of calcification have been variously described as small, nano-crystals, very fine, or granular (e.g. Wainwright, 1964; Constantz, 1986; Cohen et al., 2001; Clode and Marshall, 2003). Selective analyses of centers of calcification and adjacent fibers indicate that centers of calcification are also compositionally distinct. Several elements, notably Mg, Sr, S, Ba, and N, are enriched in the centers of calcification (e.g. Cuif et al., 2003; Gagnon et al., 2007; Meibom et al., 2004, 2006, 2007) and several isotope ratios, such as δ13C, δ18O, and δ11B, are depleted in centers of calcification (e.g. Adkins et al., 2003; Rollion-Bard et al., 2003; Meibom et al., 2006; Blamart et al., 2007) relative to the surrounding fibers. These findings have been used to support the hypothesis that centers of calcification are formed by a process distinct from that responsible for the formation of fibers (e.g. Meibom et al., 2006) and, thus, the formation of aragonite by corals is a process distinct from the inorganic precipitation of aragonite from a highly supersaturated solution. Lacking from these studies, however, is a comparison between coral aragonite and synthetic aragonite precipitated from a highly supersaturated solution.

Here, results from such a study are presented. A range of imaging techniques: light microscopy, fluorescence microscopy, scanning electron microscopy (SEM) and atomic force microscopy (AFM), as well as elemental measurements using secondary ion mass spectrometry (SIMS) were employed to characterize the morphology and chemistry of synthetic (i.e. precipitated experimentally from seawater) and biogenic aragonites (i.e. precipitated by tropical corals).

2. METHODS

2.1. Synthetic aragonite precipitates

Techniques employed for precipitating synthetic aragonites from seawater were adopted from Kinsman and Holland (1969), as modified by Gaetani and Cohen (2006). The details for some of the experiments employed in this study differ slightly, so the specifics of each experiment are briefly described below.

Aragonite was precipitated from 0.45 µm filtered Vineyard Sound (Woods Hole, MA, USA) seawater (salinity 30.8–32.1). A PTFE or PET beaker containing 600 ml of filtered seawater was placed into a Lauda RE-106 isothermal bath, and stirred continuously with a PTFE stirrer. Note that initial experiments showed no effect of the plastic type on the development of a run, so later runs used transparent PET beakers (final salinities ranged from 31.3 to 32.1). A PTFE or PET beaker containing 600 ml of filtered seawater was placed into a Lauda RE-106 isothermal bath (for some experiments, concentrated seawater was prepared by placing seawater in a PTFE beaker held in a 80 °C water bath (for some experiments, concentrated seawater was prepared in a polypropylene beaker held at 60 °C—similar results were obtained with both methods) until half the mass had been lost. The evaporated seawater was used to maintain the salinity at a roughly constant value as the 0.04 M Na2CO3 solution was added (initial salinity 31.85, final salinity 31.88). During this experiment, strontium isotope spikes (84Sr and 86Sr) were introduced at separate times into the seawater mixture. These spikes are incorporated into the growing crystals and serve as a marker from which the timing and rate of crystal growth can be determined. After the first 65 ml were added, 100 µl of a 84Sr solution was added to nearly double the 84Sr concentration. After the final Na2CO3 addition, 100 µl of 86Sr was added to approximately double the 86Sr concentration. Precipitation of aragonite was allowed to proceed for an additional 24 h after pumping stopped. Precipitates were separated from solution by filtration through a 0.7 µm glass fiber filter and rinsed briefly with distilled water and ethanol. Sr isotopes were purchased from Oak Ridge National Lab.

2.1.2. Cyclic saturation states

In Experiments 2 and 3, the saturation state was cycled over the course of each run by pulsed addition of Na2CO3 to produce precipitates with alternating regions of growth formed at high and low saturation states (bands). Experiment 2 was conducted under variable salinity conditions at either 55 °C or 65 °C. A 0.01 M Na2CO3 solution was added to the beaker of seawater in two 125 ml steps, 1 day apart, at a rate of ~1 ml min⁻¹, and the solution was stirred at 120 rpm. This experiment is described in detail by Gaetani and Cohen (2006). In Experiment 3, a ~0.04 M Na2CO3 solution and evaporated seawater were added in two steps to seawater (initial salinity 31.3, final 32.1) held in a PET beaker in a 25 °C water bath. The solution was stirred continuously at 120 rpm. 84Sr, 86Sr and 137Ba isotope spikes were added at different times over the course of each run. Pumping durations, volumes, times of isotope spike addition and solution chemistry are given in the electronic annex Table EA1, Fig. EA1 and EA2.

2.1.3. Effect of pumping rate on morphology

To examine the effects of saturation state on the morphology of precipitates, Experiments 4, 5, 6 and 7 were conducted using different pumping rates to generate different ‘steady state’ conditions under which the bulk of the precipitate formed in each experiment. In Experiments 4, 5 and 6, a syringe pump was used to continuously add a ~0.04 M Na2CO3 solution and evaporated seawater to seawater in a PTFE beaker (final salinities ranged from 31.4 to 32.8). Experiments 4–6 were conducted at 25 °C while stirring at 120 rpm. Pumping rates for the bulk of each experiment were: high = 360 ml h⁻¹ for Experiment 4, intermedia-
ate = 2 ml h$^{-1}$ for Experiment 5, and low = 0.2 ml h$^{-1}$ for Experiment 6. Injection volumes ranged from 76 to 181 ml. Aragonite seed crystals (fish otoliths, cleaned by sonication and ground to 5–300 μm) were added to Experiment 6 in an effort to speed nucleation. Precipitation was allowed to continue for 21–35 h after pumping stopped.

In Experiment 7, a ~0.04 M Na$_2$CO$_3$ solution and evaporated seawater were added continuously at a rate of 0.4 ml/h to seawater (initial salinity 31.3, final 33.5) held in a PET beaker in a 25 °C water bath. The solution was stirred continuously at 120 rpm. Isotope spikes ($^{84}$Sr, $^{86}$Sr, and $^{137}$Ba) were added at different times over the course of the run. Pumping durations, volumes, times of isotope spike addition and solution chemistry are given in the Table EA1, Fig. EA1 and EA2.

2.4.1. Growth conditions of the synthetic aragonites

Solution pH (NBS scale) was monitored throughout aragonite synthesis Experiments 1, 3, 4, 5, 6, and 7 (Table 1, Fig. 1a, Electronic annex Table EA-1, Fig. EA-1). Saturation state was also determined periodically by measuring alkalinity and calcium concentration, data are presented in Fig. 1a, Table EA-1 and Figure EA-1. Solution pH increased as Na$_2$CO$_3$ was added until nucleation occurred. Following nucleation, solution pH fell to a quasi-steady state value related to pumping rate (Fig. EA-2). At the end of each experiment, when pumping of the Na$_2$CO$_3$ solution ceased, the pH of each experimental solution dropped but remained above the initial value (~8.0). Saturation states follow a trend similar to that seen in pH data (Table EA-1). Solution chemistry for some of the runs and starting materials are provided in Table EA-1. Raman spectra of the precipitates reveal a peak at ~705 cm$^{-1}$, consistent with the presence of aragonite (e.g. Clarkson et al., 1992). Raman data are presented in Fig. EA-3.

2.2. Coral samples

The skeletons of three coral species Diploria labyrinthiformis (brain coral), Porites lutea and Porites solida were examined. D. labyrinthiformis (sample #BER002) was collected live from John Smith’s Bay, Bermuda at a depth of 13 m (Cohen et al., 2004). Porites lutea (sample #JA4) was collected live from Johnston Atoll, north central Pacific (Cohen et al., 2001). Porites solida (sample #141-B05-53) was provided by Dr David Barnes (AIMS). Porites solida was collected live in June 1989 from the Great Barrier Reef, Australia (151°E, 21°S) (Cohen and Hart, 1997).

2.3. Polishing of synthetic and coral aragonites

All samples were placed inside 2.5 cm Al rings and imbedded in Epo-thin epoxy (Buehler) prior to polishing. Double sided adhesive tape (Buehler) was used to hold specimens and rings in place as epoxy was added. Corals were prepared by breaking ~1 cm$^2$ sections of coral skeleton, roughly parallel to the axis of growth, and placing the sections on the adhesive. Synthetic aragonites were mounted by applying a light dusting of grains to the adhesive. Polishing was done on nylon cloths with alumina (Mark V Laboratories) suspended in heptane (Alfa Aesar), except for the coral used for light microscopy and SIMS analysis, which was prepared commercially (OMNI Laboratories). A small amount of water was used to initially apply the alumina grit. Each specimen was polished through a range of grit sizes down to 0.3 μm, or 0.05 μm for specimens imaged with Atomic Force Microscopy. Following mechanical polishing, a final polish with 0.02 μm colloidal silica was applied to all samples, with the exception of those used in the acridine staining experiments (see below). Colloidal silica is a water-based polishing compound, hence there is dissolution of the aragonite in addition to mechanical abrasion. An initial comparison of different techniques revealed that a colloidal silica etch on the synthetic grains gave good AFM images (Fig. EA-4). In addition to the dissolution induced by the colloidal silica, corals were etched for 30–50 s prior to AFM imaging using 0.1% formic acid and 3% gluteraldehyde in water (Cuif et al., 2003). Etching was stopped by rinsing with water. This additional etching step improved contrast between features in coral samples, as observed in preliminary comparisons of different techniques (Fig. EA-5). Samples for SEM imaging were either mounted on SEM stubs without any further preparation, or were polished as above and etched for 10s with 1 N HCl.

2.4. Imaging

2.4.1. Light microscopy and scanning electron microscopy (SEM)

Polished sections of corals and synthetic aragonites were examined in reflected and transmitted light with a Nikon Eclipse E 600 Polarizing microscope equipped with a Spot Insight color CCD camera. SEM imaging of Au/Pd coated samples (both polished and unpolished) was done using a JEOL 840 scanning electron microscope. Accelerating voltage was 15 kV unless otherwise specified.

2.4.2. Acridine orange staining

Acridine orange staining of polished sections of corals and synthetic aragonites was carried out following the protocol of Stolarski (2003). Acridine orange is a dye used to stain coral skeletons and regions retaining acridine orange are thought to have high concentrations of organic materials. Corals were etched prior to staining by placing samples in distilled water overnight to lightly etch the surface. Sam-
Fig. 1. Precipitation conditions, light microscopy images, isotope ratios, and SEM images for synthetic aragonite grains formed in Experiment 1. (a) Saturation state (open triangles) and pH (black circles) over the course of Experiment 1, the time at which pumping stopped is indicated by the cross, the times at which isotope spikes were added are indicated by vertical lines labeled with the isotope added. Errors are small relative to symbol size. (b) A grain with ionprobe spots (s) near the center (c), and in a fibrous region (f) imaged with reflected light. (c) The same grain shown in b, imaged with cross-polarized transmitted light, which better shows the fibers (f). (d) Sr isotope ratios determined for the same spots used for M/Ca data in Fig. 4. Sr84/88 ratios (black circles) are significantly higher in the fibers than the centers (p < 0.01), while Sr86/88 ratios (open triangles) show no significant difference. Points are means, error bars are standard error. (e) SEM image of a grain formed in Experiment 1, which shows fibers (f) radiating out from a granular center (g). Accelerating voltage for this image is 6 kV.

Preliminary samples were overlain with a 1% acridine orange (Alfa Aesar) aqueous solution, allowed to sit for 5 min, briefly rinsed, blotted dry and imaged on a Zeiss Axiovert inverted microscope using a mercury vapor UV source and FITC short pass filter set. Images were captured using either a Sony color CCD camera or a Canon Digital Rebel XTi camera.
2.4.3. Atomic force microscopy (AFM)

AFM imaging of polished sections of corals and synthetic aragonite was conducted at The University of Western Ontario using a Veeco MultiMode AFM and at Woods Hole Oceanographic Institution using a Veeco Dimension 3100 AFM equipped with silicon nitride tips (Veeco NP-S, with a ~10 nm radius of curvature, and BudgetSensors, with a ~20 nm radius of curvature, respectively). All images were acquired in contact mode. Image capture and processing were performed using Digital Instruments NanoScope software.

2.5. Secondary ion mass spectrometry (SIMS ion microprobe)

M/Ca (Mg/Ca, Sr/Ca, and Ba/Ca) ratios of the coral *Porites lutea* and synthetic aragonite formed in Experiment 1 were analyzed with a Cameca 3F Ion Microprobe. Following a 3-min pre-burn to remove the gold coating, a single spot on the coral sample was illuminated with the primary ion beam while measuring secondary ion intensities for 24Mg, 84Sr, 86Sr, 88Sr, 138Ba and 40Ca (Gaetani and Cohen, 2006). A 2.5 nA O-primary ion beam, ~10 µm in diameter, was accelerated at 12.7 keV. Secondary ion intensities were measured using a ~80 eV offset from the peak of the energy distribution. This energy filtering reduces molecular interferences to ~0.1% (Hart and Cohen, 1996).

In addition to the M/Ca ratios, 84Sr/88Sr and 86Sr/88Sr ratios were also determined in the synthetic aragonite grains in order to locate the isotope spikes. Individual synthetic aragonite grains were targeted with a 4nA O-primary ion beam, ~7 µm in diameter, accelerated at 12.7 keV. Secondary ion intensities (24Mg, 84Sr, 86Sr, 88Sr, 138Ba and 40Ca) were measured using a ~90 eV offset from the peak of the energy distribution. Ion probe intensity ratios were converted to molar ratios using the carbonatite standard OKA, which was assumed to be homogeneous with a Mg/Ca ratio of 4.47 mmol/mol, a Sr/Ca ratio of 19.3 mmol/mol and a Ba/Ca ratio of 1.61 mmol/mol (Gaetani and Cohen, 2006). At least eight measurements of the OKA standard were made each day samples were measured, average intensity ratios measured at the time of coral data collection were: 0.202, 2.62 and 0.16 for 24Mg/42Ca, 88Sr/42Ca and 138Ba/42Ca, respectively; at the time of synthetic aragonite measurements, values were: 0.0018, 0.014 and 0.00072 for 24Mg/40Ca, 88Sr/40Ca and 138Ba/40Ca, respectively. In all cases, standard errors were less 3%. Means were compared using a t-test (Zar, 1984).

3. RESULTS

3.1. Synthetic aragonite

The individual grains formed in Experiment 1 are roughly circular; in polarized light, the center (c) of each grain is distinguished by a dark region (Fig 1c). Radiating out from this center are aragonite fibers (f). The morphology of the grains is consistent with the spherulitic morphology found in a range of minerals i.e. a radially disposed array of acicular crystals that emerge from a common center or nucleation region (e.g., Cross, 1891; Iddings 1891), typical of crystals formed rapidly from a supersaturated or supercooled solution (Lofgren, 1971; Sunagawa, 1987; Lowenstam and Weiner, 1989). Using SEM (Fig 1e), it can be seen that the material found in the center is sub-micron in size and has a granular appearance, while the fibers (f) radiating out from the center are ~1 µm wide and several microns long.

3.1.1. Timing of growth

The Sr isotope ratios in the center of each grain formed during Experiment 1 reflect natural abundances (Fig. 1d), indicating that the centers formed prior to the addition of the 84Sr isotope spike. The 84Sr spike is present in the fibrous aragonite between the centers and the edge of the spherulite, indicating that fibers formed after the centers but prior to the addition of 86Sr (Fig. 1d). High 86Sr was found only at the edges of a few spherulites, indicating that very little aragonite precipitated following the addition of 86Sr (data not shown).

The presence of spikes allows the crystal morphology to be correlated with the solution chemistry. In Experiment 1, solution pH increased as Na2CO3 was added until nucleation occurred (Fig 1a). The granular centers of the aragonite grains formed during this high pH period (pH ~9.2), as indicated by the absence of the 84Sr spike. Following nucleation, solution pH fell to a quasi-steady state value related to pumping rate. Fibrous aragonite grew during this period when pH was ~8.9, as indicated by the presence of the 84Sr but absence of 86Sr spikes (Fig. 1b–d). Very little aragonite was deposited near the end of the experiment after pumping stopped when pH was ~8.6, as indicated by the scarcity of elevated 86Sr.

3.1.2. Stepped pumping produces banded spherulites

Fig. 2a represents a cross section through a composite of three synthetic aragonite grains formed in Experiment 2 at 65 °C. Fig. 2b shows a cross section through a single synthetic aragonite grain formed in Experiment 3 at 25 °C. The two pumping cycles used in each of these experiments lead to two cycles of high and low saturation state over the course of aragonite precipitation. The synthetic aragonite grains precipitated in these experiments are similar to those in Fig. 1c, but an additional feature, a single dark band running perpendicular to the axis of fiber growth, is present (Fig. 2a and b). In these spherulites, the dark band is located ~15 µm from the outer edge of each grain formed in Experiment 2, and ~4 µm from the edge for grains formed in Experiment 3.

Fig. 2c shows a fluorescence image of a synthetic aragonite grain, grown in Experiment 2 by cyclic pumping, stained with acridine orange. Addition of acridine orange results in increased fluorescence associated with the center (c) and dark bands (arrow) observed in light microscopy.

Fig. 2d shows an SEM image of a polished, HCl etched aragonite grain formed in Experiment 3 by cyclic pumping. The central region is composed of granular to finely fibrous material. Fine fibers radiate out from the center and become larger till reaching the band (arrow). Following the band, fibers again radiate out to the edge of the aragonite grain.
3.1.3. SEM imaging

SEM images of synthetic aragonites precipitated in Experiment 4 (Fig. 3a), 5 (Fig 3b), 6 (seeded, Fig. 3d) and 7 (Fig. 3c) at high (Fig. 3a), medium (Fig. 3b), and low (Fig. 3c and d) pumping rates reveal a systematic change in crystal morphology with pumping rate, and thus pH (Fig. EA 1,2). Synthetic aragonite grains formed in the high pumping rate run (pH ~9.5) lack well-defined crystals, and are composed of very fine fibers (Fig. 3a). Synthetic grains grown at intermediate pumping rates (pH ~8.8) are composed of well-defined aragonite blades, 1–2 μm wide and several microns long and there are clear grain boundaries between individual fibers (Fig. 3b). Synthetic aragonites precipitated in the low pumping rate run (pH ~8.2 for Fig. 3c, ~8.3 for Fig. 3d) are composed of broad (~2 μm wide), highly faceted fibers that are widely separated (Fig. 3c and d). The fibers emerge from a common center, as shown in Fig. 3c and the inset in Fig. 3d.

3.1.4. AFM imaging

AFM height images of synthetic aragonite grains etched with colloidal silica show similar changes in crystal morphology with pumping rate to those seen with SEM (Fig. 4). In all images, height is on a scale of 0–400 nm, with the highest regions shown as white, lowest as black. The length-scale, in microns, is shown on the x and y axes of each image.

In Fig. 4a, an AFM image of two synthetic aragonite grains precipitated with two stepped additions of sodium carbonate (Experiment 2) is shown. The grains have roughly circular centers (c) (only partly visible at the base of the image) surrounded by two layers of fibrous aragonite (f). A darker region of granular material, ~10 μm wide (arrow), separates the inner and outer fibrous layers. Granular materials are sub-micron in size, while fibers are micron scale features—typically 8 μm long, and 0.7 μm wide.

Fig. 4b shows a synthetic aragonite formed in a high pumping rate run (pH ~9.5, Experiment 4). The entire sur-
face of the grain is rough and granular (g), lacking defined fibers. In Fig. 4c, a synthetic aragonite formed in a low pumping rate (pH ~8.3, Experiment 6) run is imaged. Granular material (g) is restricted to near the center of
the grain. The bulk of the grain is made up of broad well-defined fibers (f) that emerge from the granular materials and radiate outward to the edge (Fig. 4c).

### 3.1.5. M/Ca ratios

Mg/Ca, Sr/Ca, and Ba/Ca ratios in centers and fibers of synthetic aragonite grains formed in Experiment 1 were measured by SIMS ion microprobe (Fig. 5). Discrete analyses of centers of synthetic grains were possible with the 7 μm diameter analytical spot. Similarly, discrete analyses of aragonite fibers emerging from the centers of synthetic aragonite grains were made. The Mg/Ca and Ba/Ca ratios are higher in the centers of the grains (6.35 ± 0.09 and 0.022 ± 0.001 mmol/mol, respectively) than in the surrounding fibers (5.01 ± 0.07 and 0.013 ± 0.001 mmol/mol, respectively) (p < 0.01). Sr/Ca ratios show no significant difference between centers (10.2 ± 0.1 mmol/mol) and fibers (10.1 ± 0.1 mmol/mol).

### 3.2. Coral aragonite

#### 3.2.1. Light microscopy

In Fig. 6a, the arrangement of aragonite crystals in a coral skeleton is seen in a cross section through a symapticum or horizontal cross-bar of *Porites solida*. In polarized light, the center of calcification (c) appears dark with poorly defined edges. Radiating outward from the center of calcification are aragonite fibers (f). This observation is consistent with the description of coral sclerodermites (Wells, 1956). In this section, the fibers are interrupted by fine dark bands (arrow), ~2 μm apart and aligned perpendicular to the axis of crystal extension.

#### 3.2.2. Fluorescence imaging

Fig. 6b shows a fluorescence image of a section of the coral *Diploria labyrinthiformis* stained with acridine orange. There is an increase in fluorescence associated with the centers of calcification (c) and dark bands (arrow) following acridine staining. This observation is consistent with that of Stolarski (2003).

#### 3.2.3. SEM imaging

Fig. 7 shows SEM images of dissepiments (horizontal sheets) in the skeletons of *Diploria labyrinthiformis* (Fig. 7a) and *Porites lutea* (Fig. 7b). The dissepiments are composed of two layers (as identified by Barnes, 1971). The primary (base) layer is built of small granular materials (g), <1 μm diameter. The secondary layer is composed of broad blade-like fibers (f), each ~1–2 μm wide and several microns long.

#### 3.2.4. AFM imaging

In Fig. 8, AFM height images of skeletal cross sections of *Diploria* (Fig. 8a and c) and *Porites* (Fig. 8b,d) reveal centers of calcification as regions of low relief (dark) and bands of aragonite fibers as regions of higher
relief (light). Fine dark bands that transect the aragonite fibers are also visible as regions of low relief. Materials occupying centers of calcification (Fig. 8d) and dark bands (Fig. 8c) are small and granular in texture. Conversely, in both species, the light bands are packed with larger elongated fibers.

In Fig. 8a, a cross section through a *Diploria* septotheca, the direction of vertical growth is from the lower right to the upper left of the image. Centers of calcification (c) appear crescent-shaped, and form a line of discrete dark crescents up the middle of the septum. Centers of calcification are continuous with fine dark bands (arrow) that extend toward the edge of the septotheca. The width of the fine bands decreases with distance from the centers of calcification. Near the centers of calcification, the fine bands are 5.3 ± 0.4 \( \mu m \) (n = 10) wide. At the outer edge of the septotheca, the width of the bands is not discernible. As the width of the dark bands decrease, the distinction between light fibrous bands and the dark bands that interrupt them becomes less distinct. Near the center of the septotheca, the transition between light fibrous bands and fine dark bands is abrupt. Toward the edge of the septotheca, the fibers appear continuous, cutting across dark bands.

In the *Porites* septum, the centers of calcification are oval rather than crescent shaped (Fig. 8b). Fine dark bands (arrows) are present in the fibers but these are not as clearly defined in this *Porites* specimen as they are in the *Diploria* skeleton.

### 3.2.5. M/Ca ratios

Selective analysis of Mg/Ca, Sr/Ca, and Ba/Ca ratios in centers of calcification and surrounding fibers of a *Porites* skeleton are shown in Fig. 9. Mg/Ca, Sr/Ca, and Ba/Ca ratios are higher in the centers of calcification (4.91 ± 0.02, 8.98 ± 0.03, and 0.0058 ± 0.0001 mmol/mol, respectively) than in adjacent fibers (3.88 ± 0.01, 8.63 ± 0.03, and 0.0034 ± 0.0001 mmol/mol, respectively). For all M/Ca ratios measured, the value for the centers was significantly higher than for the fibers (p < 0.01).

### 4. DISCUSSION

#### 4.1. Synthetic aragonite

Synthetic aragonite crystal morphology and composition are coupled to the pH and saturation state of the fluid from which the crystals grew. Within a single spherulite, the centers packed with sub-micron sized granular materials form when the saturation state of the fluid is very high. Growth of fibrous crystals outward from the centers occurs when the saturation state of the fluid has decreased following nucleation. The supersaturation achieved prior to nucleation depends on the rate at which Na\(_2\)CO\(_3\) is added; the faster the addition of Na\(_2\)CO\(_3\), the higher the saturation state achieved prior to nucleation, consistent with the work of Prieto and others (1989, 1994). In addition, the morphology of the spherulite (i.e., open, coarse versus closed, fine) and the size and shape of the aragonite needles within the spherulites change systematically with the pH (saturation state) of the seawater in which they grew. In the high pH, high saturation state experiments, fine closed spherulites form that contain densely packed fibers with ill-defined grain boundaries (Fig. 3a). Conversely, spherulites formed at low pH are typically open and coarse, containing fewer, broad, faceted fibers (Fig. 3b). This observation is consistent with systematic variations in crystal morphologies observed in non-CaCO\(_3\) minerals and polymers with increasing degrees of supercooling or with increasing supersaturation (e.g., Keith and Padden, 1963; Lofgren, 1971, 1974; Chernov, 1984; Sunagawa, 1987), and reflects the crystal morphology that allows the maximum growth rate under those conditions (e.g., Tiller, 1964). The formation of smooth sides and development of facets in the lower pumping rate experiments likely reflects a change in growth mechanism, with a rough interface associated with spherulitic growth transitioning to a smooth interface dominated by dislocation growth controlling step flow on faceted crystals (e.g. Sunagawa, 1981, Sunagawa, 1987; Prieto et al., 1989).
Measurements of Mg/Ca, Sr/Ca, and Ba/Ca show that the Mg/Ca and Ba/Ca ratios are significantly elevated at the center of the spherulite relative to the fibers (Fig. 5). In each experiment, the highest saturation state occurs at the onset of nucleation, thus the center of each spherulite contains crystals expected to have the highest growth/preparation rate (Burton and Walter, 1987) (though it should be noted that growth mechanism and relative areas of different crystal faces also change, and could influence composition as well). This relationship between crystal growth rate and M/Ca ratio is consistent with the growth rate dependence expected for Mg/Ca and Ba/Ca, as all M/Ca ratios are expected to increase with higher growth rates due to more efficient entrapment of an impurity enriched mineral surface layer composition at higher crystal growth rates (Watson 2004; Gaetani and Cohen, 2006). Gabitov et al. (2006, 2008) showed that the growth dependence of Mg/Ca in aragonite is much higher than that of Sr/Ca. Therefore, the absence of a significant elevation of Sr/Ca in the centers found in this study is consistent with their data.

4.2. Coral aragonite

Data presented here concur with earlier observations that coral sclerodermites consist of aragonite needles radiating out from regions of fine granular materials or ‘nanocrystals’ (Figs. 6–8) (Vaughan and Wells, 1943; Wainwright, 1964; Constantz, 1986; Cohen et al., 2001; Clode and Marshall, 2003). The sub-micron-sized granular materials are found at the base of dissepiments (Fig. 7a,b), in the centers of calcification (Fig. 8) and in the fine bands that cut across aragonite fibers (Fig. 8).

Regions associated with granular materials are also associated with an increase in fluorescence following acr-
dine orange staining (Fig. 6b), which is consistent with the results of Gautret et al. (2000) and Stolarski (2003). Mg/Ca, Sr/Ca, and Ba/Ca ratios are found to increase at the centers of calcification relative to adjacent fibers (Fig. 9), which is consistent with the findings of Meibom et al. (2004, 2006).

4.3. Results of inorganic precipitation experiments in relation to coral skeleton morphology and composition

Aragonite crystals formed by experimental precipitation from a supersaturated seawater solution and the aragonite crystals formed by living corals during skeletogenesis share several morphological and compositional features. Both the synthetic aragonites precipitated in this study and the coral sclerodermites are composed of two distinct types of crystals: sub-micron-sized granular materials and larger elongate fibrous crystals that radiate out from the granular materials. Granular materials are found near the centers of synthetic grains, in the centers of calcification of corals, and in dark bands found both in corals, and in the synthetic aragonites formed by stepped additions of Na₂CO₃. Higher Mg/Ca and Ba/Ca ratios and acridine orange staining correspond to regions of granular materials in both synthetic aragonite (Fig. 2c, 5) and corals (Fig. 6b, 9).

Since the composition and morphology of crystals provide insights into the conditions under which they grew (e.g., Lofgren, 1971; Reddy and Nancollas, 1976; Marsh, 1988; Prieto et al., 1997; Marsh, 1998; Cohen and McConnell, 2003; Tong et al., 2004; Wasylenki et al., 2005), the observed crystal morphologies and compositions associated with known conditions in the synthetic experiments may be useful in assigning possible conditions of formation of naturally formed precipitates.

The similarity between the finely fibrous to granular materials at the centers of spherulites (Fig. 4a), grains formed at very high pH (Fig. 4b) and materials occupying centers of calcification, fine bands, and the base layer of dissolutions in coral skeletons (Fig. 8), suggests that these regions in the coral represent material formed at substantially elevated saturation states. The morphology of fibrous aragonite in coral skeletons (Fig. 7a and b) is consistent with that of aragonites grown in the mid pumping (moderate precipitation) rate experiment (Fig. 3b): tightly spaced but with distinct boundaries between fibers. This may suggest that fibrous growth in corals occurs at a saturation state below that needed to induce nucleation but substantially above that of ambient seawater.

4.3.1. Band formation in corals and synthetic aragonite

The formation of alternating bands of fibrous crystals and granular materials in synthetic aragonites precipitated in Experiments 2 and 3 resulted from the stepped addition of Na₂CO₃ which caused the saturation state of the seawater solution to cycle during the experiment. Addition of the first volume of Na₂CO₃ elevated the solution pH (supersaturation), initiating nucleation and the formation of granular materials at the center of the spherulites. Following nucleation, the solution pH dropped, enabling fibers to grow and radiate outward from the center. Addition of a second volume of Na₂CO₃ solution elevated the solution pH, favoring nucleation over elongation of pre-existing crystals and forming a fine band of granular materials following again by radial growth (Fig. 4a).

Such a cycling of fluid saturation state may explain the formation of microscale bands of alternating fibers and granular materials within the coral skeleton (Jell, 1974; Soroauf and Jell, 1977; Risk and Pearce, 1992). The coral crystal morphologies reported here (Fig. 8a-c) are consistent with repeated cycles of high and moderate saturation states. Variations in rates of ion pumping or internal fluid flow could generate both spatial and temporal variations in saturation state. Zoanthellate coral calcification rates (e.g. Kawaguti and Sakamoto, 1948; Goreau, 1959; Barnes and Chalker, 1990), and the internal pH of the coral (Al-Horani et al., 2003), are both known to change substantially over light dark cycles. Thus, the microscale bands (often called daily growth bands) within zoanthellate coral skeletons may be the product of daily cycles in saturation state.

A model to explain the banding patterns seen in Diploria is presented in Fig. 10. In Diploria, the banding patterns are characterized by thick regions of granular material near the center of a skeletal element, with a gradual thinning of the granular band away from the center, transitioning to fibrous growth and a loss of bands (Fig. 8a). This pattern could be explained by the presence of a highly supersaturated fluid adjacent to where the granular centers form, transitioning to a moderate saturation state far from the centers, where fibers are continuous. The region of highest saturation state would be associated with the fastest growth and, thus, the thickest band of granular material. Moving down that saturation state gradient, growth would slow, shifting from granular to fibrous material. At different times, a more uniform, moderate, saturation state could exist throughout the calcifying environment, generating a band of fibrous crystals.

The proposed cycle in saturation state could account for both the high Mg/Ca ratios at centers of calcification, and the alternating micron-scale bands of high and low Mg/Ca ratios reported by Meibom et al. (2004). This is consistent
with the suggestion of Tsukamoto and Tsukamoto (1996) that growth rate variation could account for some of the variability in Mg/Ca ratios and is similar to the explanation proposed by Eggins et al. (2004), which attributes daily Mg/Ca bands in foraminifera to cycles in pH.

Cycles in the saturation state of the calcifying environment can also give rise to fine-scale heterogeneity in isotope ratios. Differences in $\delta^{13}C$ and $\delta^{18}O$ between centers and fibers, as well as fine-scale heterogeneity in the composition of coral fibers may, in part, be due to diffusion kinetics favoring the lighter isotope in the faster growing regions (Rollion-Bard et al., 2003; Meibom et al., 2006). The calcification model proposed by Adkins et al. (2003) expands upon the diffusion model to incorporate multiple carbon sources which contribute to skeletal $\delta^{13}C$ in a pH dependent manner. Similarly elevations in the pH of the calcifying fluid induce depletion of $\delta^{18}O$. This model is compatible with the compositional response of aragonite precipitates to variations in saturation state proposed here. In the Adkins et al. (2003) model, high pH in the calcifying environment is associated with light $\delta^{13}C$ and $\delta^{18}O$ and rapid growth rate. Assuming that saturation state of the precipitating fluid is linked to pH, centers of calcification and bands of granular crystals should have a lighter $\delta^{13}C$ and $\delta^{18}O$ signature relative to fibers.

Some independent observations appear to contradict the proposed model. For example, Blamart et al. (2007) show that centers of calcification have lower boron isotope ratios than adjacent fibers, which the authors interpret to mean that crystals in centers of calcification form at a lower pH than the surrounding fibers. However, factors controlling the boron isotope ratios of coral skeletons are not well-understood and considerable variability has been found between different coral species, even when grown under similar environmental conditions (e.g. Vengosh et al., 1991; Pagani et al., 2005; Klochko et al., 2006, 2009; Xiao et al., 2006; Blamart et al., 2007). In two separate studies, Clode and Marshall (2002) and Tambutté et al. (2007) examined the interface between the skeleton and the calicoblastic ectoderm, and found, in freeze-fractured specimens, that the membrane lays flush with the skeletal surface with the possible exception of nanometer scale spaces. Tambutté et al. (2007) interpreted this as the absence of a reservoir in which saturation state could be regulated, however that nanometer scale volumes are sufficient for saturation state to control crystal formation cannot be ruled out.

The results presented here show that features of coral skeletons such as: banding patterns, fibrous and granular materials, Mg/Ca ratios, and acridine fluorescence patterns, can be reproduced by manipulating the saturation state. Our results suggest that these features cannot be used as firm evidence of organic materials (e.g., templates) controlling crystal growth. Cycles in saturation state may not be the only factor controlling the morphology and chemistry of coral crystals. By using synthetic aragonite spherulites as a control material, it may be possible to identify coral features that cannot be reproduced by manipulating saturation state alone and, thus, may be indicative of biological control over and above controlling saturation state within the calcifying environment.

4.4. Differences

Despite the numerous similarities between synthetic aragonites and coral skeletons, there are some key differences. The most significant of these for sample preparation is the greater apparent solubility of the synthetic aragonites (Fig. EA 4.5). This may be due to coral-derived organic materials decreasing the solubility of the coral skeleton or it may be a reflection of the much smaller size of individual spherulites (<100 µm diameter for a spherulite, versus millimeters for a coral), making them more vulnerable to dissolution.

5. SUMMARY

The morphology and composition of synthetic aragonitic spherulites precipitated experimentally from seawater is shown to change systematically with the pH (saturation state) of the precipitation environment. Spherulites progress from coarse open structures at pH values near seawater to fine closed structures at high pH. The highest pH precipitates have a granular appearance, similar to the
granular appearance of centers of calcification and bands in corals, whereas coral fibers resemble synthetic fibers grown at lower pH. Variations in elemental ratios coincide with the morphological variations, suggesting that the saturation state influences both the morphology and composition of the precipitate. The similarities observed between portions of the coral skeleton and synthetic aragonites grown under varying controlled chemical conditions may prove useful in interpreting the coral biomineralization process. These observations suggest that the saturation state beneath the calcifying environment which lead to the formation of centers of calcification and fine bands within the coral skeleton.

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APPENDIX A. SUPPLEMENTARY DATA


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