

Skeletal growth controls on Mg/Ca and P/Ca ratios in tropical Eastern Pacific rhodoliths (coralline red algae)

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ARTICLE INFO

Keywords:

Rhodolith

Mg/Ca

P/Ca

Paleoclimatology

Paleotemperature proxy

ABSTRACT

The Mg/Ca ratios of non-geniculate coralline red algae (CA) are often assumed to be solely dependent on the sea surface temperature (SST) in which they grow, suggesting that they are ideal proxies for SST reconstruction. Mg/Ca ratio cyclicity is also commonly used as a marker for annual growth band identification in many CA species. However, other controls on elemental uptake such as light, calcification mechanisms, and vital effects may control elemental ratios (e.g. Mg/Ca and P/Ca) in certain genera or species of CA, thus complicating the use of Mg/Ca and P/Ca ratios as a proxy source for SST and paleo-environmental reconstructions. Here we present evidence showing that the Mg/Ca and P/Ca ratio cyclicity in unattached living tropical *Lithothamnion* sp. ($n = 8$) rhodoliths correlate $> 90\%$ to the number of sub-annual growth bands ($n = 1$ to 20) formed during a 172-day controlled growth experiment and not to temperature or other environmental conditions (e.g. salinity, pH, etc.). This pattern is also observed in the wild, pre-experimental growth further supporting that the bands are not annual. The Mg/Ca_{alga} ($R^2 = 0.67$; p -value = 0.013; $n = 8$) and P/Ca_{alga} ($R^2 = 0.64$; p -value = 0.018; $n = 8$) significantly correlate to skeletal extension during the experiment. This work has implications for trace elemental proxy development using CA as well as the influence of skeletal extension on elemental incorporation in biogenic carbonates, particularly rhodoliths of *Lithothamnion* sp. from the Gulf of Panama, Panama.

1. Introduction

Rhodoliths are a free-living form of coralline red algae (CA) that grow discoidal to ellipsoidal to spheroidal morphologies (Boscence, 1983) up to 20–30 cm in diameter and roll on the seafloor (Adey and Macintyre, 1973). In comparison, encrusting forms of CA attach to seafloor substrate or act as a binding surface on reefs and can grow to a similar thickness (e.g. *Clathromorphum nereostratum* at 20 cm thick; Adey et al., 2013). Both forms predominantly produce high-Mg calcite (HMC) skeletons and incrementally accrete bands through extension in individual protuberances (rhodoliths; Fig. 1C) or in non-branching layers of the thallus (encrusters). Upon accretion, variable trace elemental concentrations are recorded in the crystalline and organic matrices that can potentially be used for reconstructing the paleoclimatology, paleoenvironmental, or paleoupwelling history for the area in which the organism grew.

One fundamental way of examining skeletal extension is by understanding the growth bands accreted by the CA. Halfar et al. (2000) studied the physical components of banding in rhodoliths and identified

two tiers of banding were present using elemental maps of Mg/Ca: This technique revealed that first-tier banding is represented by variations in light and dark calcified layers that can then be sub-divided by second-tier, or thinner bands. These growth bands are present even if not visible when examined using microscopy, including SEM imaging (Halfar et al., 2000). The timing of when these bands grow is more controversial. For some species (e.g. the encrusters *Clathromorphum compactum* and *C. nereostratum*, and the rhodolith former *Lithothamnion glaciale*), banding has been demonstrated to be first-tier and annual in nature, recording valuable information for reconstructing paleoclimate and paleoceanographic records (Williams et al., 2014; Halfar et al., 2011; Hetzinger et al., 2013; Gamboa et al., 2010; Halfar et al., 2007; Kamenos et al., 2008). Halfar et al. (2000), Blake and Maggs (2003), and others have also shown that CA, particularly rhodoliths, can form sub-annual primary banding (aka more than one predominant band in a single year). Sletten et al. (2017) found *Lithothamnion* sp. samples ($n = 10$) from the Gulf of Panama (GOP) grew between 0 and 20 bands within a 172-day (~6 months) growth experiment, varying largely by their axial position to a light source. Further results from this study

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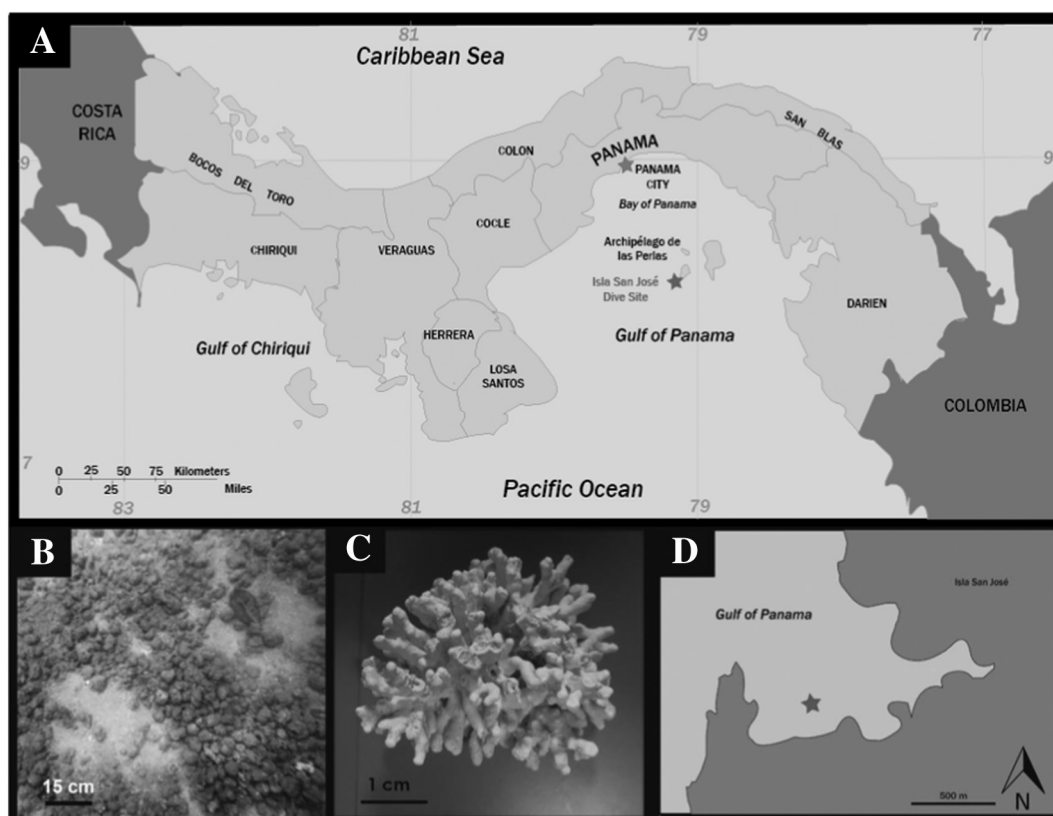


Fig. 1. (A) Map of Panama including dive collection site (lower star) and the Smithsonian Naos Marine Facility location near Panama City (upper star). Latitude (7–9°N) and longitude (77–83°W) are indicated by numbers marked on the perimeter; (B) Distribution of rhodoliths on the seafloor at 2–3 m depth; (C) Example of an open-branching GOP rhodolith sample rinsed with freshwater and air dried; (D) Map showing approximate location of sample collection, more closely approximated by the starred location.

suggested that temporal variation, temperature, salinity, pH, and nutrient loading were not predominant controls on band formation. However, rhodoliths of a different species or from different locations may respond to these factors in varying degrees.

The Mg/Ca ratios of algal skeletons have been shown to be a viable proxy for sea surface temperature (SST) reconstruction based on the endothermic substitution of Mg for Ca at higher temperatures (Hetzinger et al., 2011; Lea, 2003). Examples include CA species in the northern, boreal latitudes (e.g. *C. compactum*, *C. nerostratum*, *L. glaciale*) (Williams et al., 2014; Gamboa et al., 2010; Kamenos et al., 2008); rhodoliths in the sub-tropics (*Lithophyllum kotschyianum* f. *affine*) (Caragnano et al., 2014); and rhodoliths in the tropics (*Sporolithon durum*) (Darrenougue et al., 2013). In these species, the authors argue that the Mg/Ca ratios track concurrently with the annual SST cycles that align with annual bands. These arguments were based on staining techniques that were first used to determine annual growth bands were present, and secondly to support that elemental cyclicity for recorded Mg/Ca ratios correlated with instrumental temperature data (e.g. *C. compactum* [Halfar et al., 2008] and *L. glaciale* [Kamenos et al., 2008]). Given the correlation between banding, Mg/Ca, and SST in some CA species, spectral analysis has shown positive relationships to long-term SST variability such as the North Atlantic Oscillation (NAO) (Gamboa et al., 2010) and the Oceanic Nino Index (ONI) (Darrenougue et al., 2014) making CA a useful proxy for studies in long-term climate change.

Phosphorous to calcium ratios have not been published for CA before, but may help elucidate the study of elemental uptake in CA. P/Ca is important because it may offer a proxy for reconstructing paleo-nutrient concentrations or upwelling regimes as has been suggested in corals (LaVigne et al., 2010). In upwelling locations, increased nutrient concentration (e.g. P, Ni, and Si) in seawater occurs during upwelling seasons (Caragnano et al., 2014; Matthews et al., 2008). The GOP is an

upwelling zone (Jan-Mar) where ambient PO_{4sw} (hereafter SRP [soluble reactive phosphorus]) has been measured to be $\sim 1.2 \mu M$ in the upper layer and experiences ~ 3 fold variation from the upwelling to non-upwelling season (Apr-Dec) (D'Croz and O'Dea, 2007). LaVigne et al. (2008, 2010) found that the scleractinian coral *Pavona gigantea* from the GOP recorded P/Ca and suggested that the elemental content of the coral could be used to reconstruct seasonal upwelling in the GOP and provide a direct paleo-proxy for nutrient concentrations in the upper surface layer. Since upwelling also plays a significant role in the temperature and salinity regimes of the GOP ($\sim 15^\circ C$ and 2.5 PSU respective ranges between the two seasons), it would seem plausible that the Mg/Ca and P/Ca ratios would show a relationship to the upwelling cycle. If measurable in CA, P/Ca could potentially be used as comparative proxy to the P/Ca in corals and other biogenic carbonates for the purpose of building multi-proxy paleo-upwelling records.

Although SST is the most commonly suggested control on Mg/Ca, as well as other elemental ratios (e.g. Li/Ca, U/Ca, or Sr/Ca), (Williams et al., 2014; Caragnano et al., 2014; Adey et al., 2013; Halfar et al., 2011; Hetzinger et al., 2011; Kamenos et al., 2008; Chave and Wheeler, 1965), additional controls on growth and elemental incorporation into CA skeletons may include: physiological or biological controls known as “vital effects” (Hetzinger et al., 2011; Moberly, 1968), taxonomic affiliation (LaVigne et al., 2010; Gillikin et al., 2005), long-term Mg/Ca variation in seawater (Ries, 2006), calcification rate via light exposure (Borowitzka and Larkum, 1987; Moberly, 1968), and skeletal growth rate (LaVigne et al., 2010; Borowitzka and Larkum, 1987; Moberly, 1968). Gabitov et al. (2014) have shown that in inorganic calcites Mg/Ca partitioning occurs as a result of growth rate of the crystal. Although this level of examination has not been undertaken using biogenic carbonates, it is possible that these controls are expressed at the micro-scale (crystal nucleation) and macro-level (skeletal extension) in biogenic carbonates, which may be the case for

some species of CA. Each of these potential controls needs to be considered when assessing a new species that is under consideration for being used as a paleo-archive.

In this paper, we examine the effect of skeletal extension on the Mg/Ca and P/Ca ratios in a sample of rhodoliths (*Lithothamnion* sp.) using laser ablation inductively coupled plasma mass spectrometer (LA-ICP-MS) techniques. Additionally, we compare and contrast plausible mechanisms of elemental uptake and reasons for their correlation to sub-annual bands in rhodoliths.

2. Materials and methods

2.1. Sample collection and experimental setup

In March 2014 live rhodoliths ($n = 30$) of *Lithothamnion* sp. were collected via SCUBA diving from the Archipelago de las Perlas (Pearl Islands) located approximately 48 km off the southern Pacific coast of Panama in the GOP (08°15'.417 N; 079°08'.053 W; Fig. 1). Samples were then transferred to the Smithsonian Tropical Research Institute (STRI) (2012) Naos Marine Facility (NMF) located near the Panama Bay in Panama City, Panama. Samples were transported in seawater-filled coolers to the NMF where they were placed in covered, outdoor flow-through aquaria with circulation pumps and filtered seawater (Strain-rite polyester felt 10 μ m pore size filter bag). Samples were placed in tanks for one week prior to being stained with Alizarin red S (ARS) stain to mark the start of the 172-day experiment. Rhodoliths were stained for 60-h in 10 L of seawater mixed with 15 mg/L of ARS stain while under continuous LED lighting (Jiangjing; 18 W; 170 lx). This method was tested prior to the experiment and shown to create successful, bright pink stain lines in the rhodoliths of extra samples collected from the field site. Stained rhodoliths ($n = 30$) were then placed individually in experimental microcosms (13 cm $\times$ 24 cm white bins) to monitor growth under ambient conditions with the exception of being placed below LED lamps (Jiangjing; 18 W; 170 lx) on a 12:12 h cycle. Light intensities were monitored using a HOBO Pendant logger (Model UA-002064) and converted from lux units to photosynthetic photon flux density (PPFD) using a Licor sensor (Kipp & Zonen PQS1) calibration (Sletten et al., 2017). PPFD was then used to quantify PAR (photosynthetically active radiation) and better relate the influences of light on algal growth. Seawater was fed to each microcosm via a 1/4" plastic tube connected to a header tank system that received seawater from the flow-through aqueduct system from coastal water near the NMF providing a 100% turnover rate of the microcosm water every 20 min. Rhodoliths were overturned in random positions every two weeks when the rhodoliths were removed to clean the microcosm and remove fouling algae. For full experimental setup see Sletten et al. (2017).

2.2. Water monitoring and analyses

During the experiment several ambient seawater conditions were monitored. Temperature was recorded hourly using a HOBO Water Temp Pro v2 (Model U22-001) logger. In addition to temperature, salinity, pH, SRP, and seawater cations were measured. Of these, salinity was recorded twice a day, while the other parameters were measured on a bimonthly basis to track background conditions. This resulted in a total of 12 sample events for SRP. SRP ($n = 12$) was measured at the Dauphin Island Sea Laboratory (AL, USA) on a Skalar San + continuous flow autoanalyzer with wet chemistry modules designed for standard colorimetric techniques (SRP analyzed using EPA, 1993 Method 365.1, Rev. 2.0) (Lu et al., 2015). Cation elemental concentrations of seawater (e.g. Mg and Ca) were also monitored bimonthly throughout the experiment from the middle of May to September 2014, resulting in only 10 sample events for these parameters. Samples for elemental geochemistry were collected using trace element clean sampling protocols. Samples were spiked with Optima-

grade HNO₃ and stored in refrigerated conditions (except during shipping to the USA) in 30-mL LDPE bottles until they were analyzed. Samples ($n = 10$) were analyzed using the ICP-MS at the Trace Elemental Geochemistry Lab at Union College (NY, USA) using a Perkin Elmer Elan 6100 DRC inductively coupled plasma – mass spectrometer (ICP-MS). Samples were diluted with deionized water (> 18 M Ω /cm) to assure a salt concentration $< 0.2\%$. Calibration standards were made from stock solutions and scandium was used as an internal standard. Percent relative standard deviations on the NRC SLRS-5 certified reference material were within acceptable ranges (6.2% for Mg and 1.8% for Ca; $n = 5$).

2.3. Algal sample preparation and species identification

Ten rhodoliths were selected using a randomization function in Excel at the end of the experiment for thick-section preparation. At minimum, one branch was selected in the long [A], intermediate [B], and short [C] axes of each rhodolith, totaling 34 samples. The branches were dried, cleaned ultrasonically, mounted in Epothin epoxy resin with hardener (Buehler), and polished in thick section (1–9 mm) to ~ 6 μ m in preparation of measuring the trace elemental content of the experimental growth (i.e. post-ARS growth) and wild growth (i.e. pre-ARS growth) using LA-ICP-MS. Of this original sample set, 16 branches were randomly selected and used for high-resolution elemental analysis representing 9 of the 10 rhodoliths used to make thick sections. A summary of the counted bands for these selected branches from the study by Sletten et al. (2017) is shown in Fig. 2. Each thick section was imaged before and after laser ablation using geo.TS software and an automated Olympus VS-BX reflected light microscope at the University of Toronto – Mississauga (Canada) Paleoclimate and Geobiology lab. High-resolution imaging, SEM, and DNA analysis were used in combination to identify the samples as *Lithothamnion* sp. (Sletten et al., 2017).

2.4. LA-ICP-MS analysis

Rhodolith $^{26}\text{Mg}/^{43}\text{Ca}$ and $^{31}\text{P}/^{43}\text{Ca}$ ratios were analyzed using the LA-ICP-MS at Union College (CETAC LSX-213 frequency quintupled Nd:YAG laser ablation unit ($\lambda = 213$ nm) coupled to a Perkin Elmer Elan 6100 DRC ICP-MS) as outlined in Gillikin and Dehairs (2013). Helium was used as the carrier gas (600 mL/min), and was mixed with argon (800 mL/min) after the ablation cell. Laser settings included a 50 μ m spot size, 100% laser energy, 10 Hz shot frequency, and 10 μ m/s

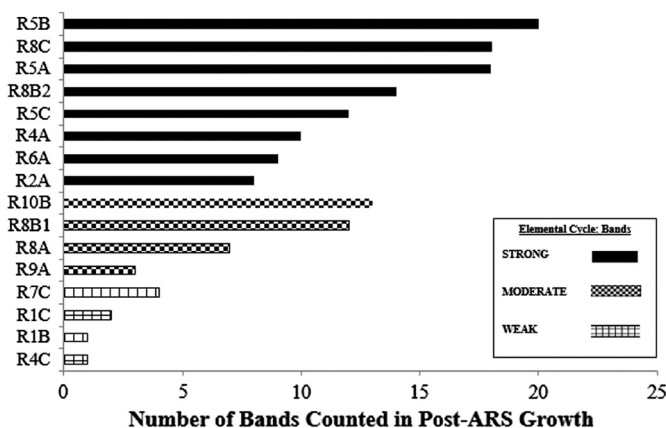


Fig. 2. Rhodolith branch samples ($n = 16$) used in this study to correlate elemental ratio cycles to growth patterns (i.e. algal banding). The patterned bars represent the strength of the relationship between the number of observed elemental cycles and the number of bands counted. The letter after the sample number (e.g. R1) indicates the branch orientation the sample was collected from. These included the long (A), intermediate (B), and short (C) axes. At least one branch from 9 of the 10 randomly selected rhodoliths from the growth experiment are represented, with some samples (e.g. R8 and R1) having more than one branch from that individual sample represented in this comparative study.

scan rate resulting in a sample collection every 0.87 s. Laser transects up to 2.8 mm in length (mean 1.4 mm) were run along the central growth axes of individual branches in the post-ARS growth ($n = 16$) in order to directly compare these data to monitored seawater conditions. Elemental data were also collected in this way in the pre-ARS growth ($n = 14$) to compare experimental results to wild growth. When axes angles changed, transects overlapped with contemporaneous sections in the previous portions of the branch so as to achieve as continuous a record as possible. For all line scans, a 15 s shutter delay was used so that each series of sample data was preceded by gas blank data, which was subtracted from the following line scan data (raw data signal to blank ratios were 1.0% for Mg and 6.5% for P). Each line was pre-ablated at a scan rate of 150 $\mu\text{m/s}$ to remove contaminants before being scanned again at a scan rate of 10 $\mu\text{m/s}$. NIST 612 was used as the calibration standard and the USGS (2011) standard MACS-3 was used to check the calibration. Calibrations and data reduction were performed by ^{43}Ca normalization and gas blank subtraction using the laser data reduction program Iolite (<https://iolite-software.com/>). MACS-3 shows acceptable reproducibility over the analysis of these samples (percent relative standard deviation (%RSD) for Mg is 3.5% and for P is 11.8%, $n = 24$ over several analytical days). These %RSD values are very similar to those reported in Jochum et al. (2012) who used different mass resolutions (low and medium) on a high resolution ICP-MS and lasers with 213 nm and 193 nm wavelengths (3.7% for Mg and 15.4% for P). However, we expect lower variability in our samples because they have higher concentrations than the MACS-3 (for example, see Gillikin et al., 2006). All trace elemental values output in ppm were converted to molar concentrations ($\text{Mg/Ca} = \text{mol/mol}$ and $\text{P/Ca} = \text{mol/mol}$).

2.5. Time series construction and correlation of elemental data to banding and growth rates

Thick section samples were re-imaged after the samples were analyzed by LA-ICP-MS in order to measure the length of each laser transect. Total length was divided by the number of data points, originally recorded in seconds, and equally distributed across the length of the laser transect. The constraints on the post-ARS growth included the ARS line, marking the start of the experiment on March 29, 2014 and the tip of the branch sample, marking the end of the experiment on September 19, 2014, totaling 172 days. It was not possible to stain or mark interim growth phases due to the algae's slow growth and bleed-over of the ARS from one band to the next, which resulted in growth strictly being measured as a total growth and no means to measure variable growth rates over time. Pre-ARS growth (growth in the wild) was measured as distance from the outer most extent of the ARS line to the end of the branch near the core of the rhodolith. Mg/Ca and P/Ca profiles were overlain on images of the rhodolith samples and aligned with the start and end of each associated laser transect to compare directly with the banding patterns. Transects were divided evenly into 10 sections to determine percent of correlation (0–100%) between elemental cycles and algal band alignment. Table 1 shows an example across 6 growth bands of the types of relationships that were observed and then designated as strong, intermediate, and weak.

Elemental data were compared to annual growth rates to determine if skeletal extension held some control on elemental uptake in the algae. Annual growth rates (i.e. rate of extension) were calculated by measuring the total amount of growth after the ARS stain line was divided by 172 days. This daily rate was then multiplied by 365 to obtain an annual rate that is more comparable to other studies. In order to compare $\text{P/Ca}_{\text{alga}}$ to SRP, the $\text{P/Ca}_{\text{alga}}$ ratios along each transect were equally divided into sets of 12 and averaged to acquire a single value so that the algal growth could be compared to the seawater chemistry, which only provided 12 data points. This yields a temporal resolution of ~ 14 d/data point. Similarly, the $\text{Mg/Ca}_{\text{alga}}$ ratios were divided evenly

into 10 groups and the values averaged so that they could be compared to the Mg/Ca_{sw} values, which only had 10 data points. For this data group temporal resolution is ~ 17.2 d/data point. This method assumes a constant growth rate.

2.6. Statistical analysis

A least squares linear regression analysis was used to correlate P/Ca and Mg/Ca to growth rate, P/Ca, and SRP, as well as $\text{Mg/Ca}_{\text{alga}}$ and Mg/Ca_{sw} . All analyses were carried out using a combination of R version 3.2.2 (<https://www.r-project.org/>) and Microsoft Excel. All significance levels, α , were set to 0.05 for all statistical analyses.

3. Results and discussion

3.1. Mg/Ca and P/Ca correlations to growth band cyclicity

For 8 of the 16 branches (50%) from 9 rhodolith samples measured using LA-ICP-MS, Mg/Ca and P/Ca ratios showed a 90–100% (i.e. strong; Table 1) relationship between the number of elemental cycles and the number of bands grown by the algae during the 172-day experiment (i.e. post-ARS growth) (Figs. 2 and 3). There were also moderate correlations (50–89% correlation; Table 1) for 4 of the 16 branches (25%) and weak correlation ($< 49\%$ correlation; Table 1) in 4 of the 16 branches (25%) between elemental cycles and number of bands grown in the post-ARS growth. In the pre-ARS growth, similar relationships were observed, where 12 out of 15 (80%) pre-ARS laser transects showed moderate to strong correlation between the Mg/Ca, P/Ca, and band frequencies. An example of these correlations in the pre-ARS growth is shown in Fig. 4. There also seems to be a relationship between the number of bands grown and the degree of correlation between the number of elemental cycles and bands. Fig. 2 shows the post-ARS bands counted for each branch analyzed. Out of the 16 branches used in this study, the 8 with the highest band count also had the strongest correlations to elemental cycles. Four out of the 5 shortest post-ARS branch tips had the weakest correlation to band cycles, and the 4 remaining branches fell in between with moderate correlations. This may suggest that branches that grow more continuously with fewer time gaps provide a more accurate record of the trace elements being recorded in the skeleton than branches with < 4 bands of growth. Thus, the more consistent growth allows for a clear correlation between the number of elemental cycles and elemental ratio cyclicity.

The amplitude of the elemental cycles in both the post- and pre-ARS growth are between 0 and 0.6 mol/mol (Mg/Ca) and 0–25 mmol/mol (P/Ca), although some portions of the pre-ARS transects observed showed diminished elemental ratios compared to the rest of the records by about 50% (Figs. 3 and 4). Early diagenesis or organic decomposition could account for reduced elemental content in the pre-ARS growth. This is interesting, considering that the *Lithothamnion* sp. samples from the GOP have been shown to likely only be about 3–5 yrs. old when taking into account the fact that they show sub-annual banding, and not 40–123 yrs. old, as originally hypothesized based on previous work done by Schäfer et al. (2011) (see Sletten et al., 2017). However, a more likely explanation may be that environmental and physical conditions, such as rhodolith turnover on the seafloor, caused reduced light exposure and therefore reduced elemental uptake during calcification processes. A comparison study using in situ rhodoliths grown in the wild and rhodoliths grown in microcosms would help elucidate this observation.

Because half of the post-ARS growth showed a strong correlation between Mg/Ca and P/Ca ratios and growth bands, in addition to 80% of the pre-ARS growth showing moderate to strong correlation, it is evident that there is a connection between how the branches grow and how the trace elements are incorporated into the skeletal structure. This perhaps suggests that both Mg and P are a part of the calcite matrix and not the organic matter interwoven in the crystal lattice. Mg is known to

Table 1

Graphical description and examples of elemental cycle patterns aligning with algal banding for the purposes of the qualitative correlation analysis. Dashed line represents the growth axis.

Relationship	Cycles:Bands	Representative Pattern
Strong	90 - 100%	
Moderate	50 - 89%	
Weak	<49%	

be a part of the calcite matrix in *L. glaciale* and *Phymatolithon calcareum* (Kamenos et al., 2009), which is likely also the case for these *Lithothamnion* rhodoliths considering their analogous HMC composition. The placement of P in the crystal structure is certainly possible given the small size of P (0.98 Å) compared to Mg (1.45 Å), and both of their ability to replace Ca (Ishikawa and Ichikuni, 1981). However, further investigation is needed to confirm the amount of P in the crystal lattice as opposed to carbonate-bound organic material.

The total number of bands grown in the post-ARS growth ranged between 1 and 20 (Fig. 2) and was shown to be positively correlated to total extensional growth ($R^2 = 0.82$; p -value ≤ 0.001). However, the

band widths on average were similar (0.15 ± 0.13 mm) showing systematic and consistent growth patterns with average pre-ARS growth bands widths (0.18 ± 0.07 mm) (Sletten et al., 2017). Sletten et al. (2017) concluded that post-ARS growth showed no direct correlation to environmental parameters (e.g. temperature, salinity, pH, etc.) and that the strongest control on extension during the experiment was the axial position of the rhodolith to a light source when placed under direct or indirect lighting. In addition to the natural variability of the branched rhodolith surface, this was also influenced by bi-weekly overturning and repositioning of the rhodoliths during the experiment, similar to what occurs in the wild. One method to simplify understanding the parameters affecting growth and better identify elemental relationships to growth patterns in future studies is to focus on attached living CA.

Here we observe Mg/Ca and P/Ca cycles that align with sub-annual bands in the post-ARS (Fig. 3) and pre-ARS growth (Fig. 4), where the peaks predominantly match up with the light bands and the troughs with darker bands. This agrees with the observations by Schäfer et al. (2011) who identified the peaks of Mg/Ca ratios in *Lithothamnion* sp. from the GOP (measured in wt% using electron dispersive x-ray techniques) to align with the lighter cell rows of the rhodolith bands. Freiwald and Henrich (1994) proposed an environmental mechanism that would potentially generate the opposite relationship, where the peaks align with the dark bands and the troughs with the lighter cells in the banding. They postulated that CA limiting nutrients (e.g. P and N) are absorbed within the dark growth bands of arctic and subarctic *Lithothamnion* sp. during colder, more nutrient rich seasons of the year. This would hypothetically produce higher nutrient to Mg ratios that correlated with the dark banding. Such a hypothesis would seem applicable to the GOP upwelling seasons when nutrient concentrations are higher in surface waters where the algae grow; however, based on the findings by Sletten et al. (2017), these GOP rhodoliths do not generate banding that align with the upwelling seasons.

Similar to Schäfer et al. (2011), Hetzinger et al. (2009) and Gamboa et al. (2010) demonstrated that high Mg values (aka peaks) occur in *C. nereostratum* and *C. compactum*, respectively, in the light-colored (less dense), summer growth. Dark bands mark dense, winter growth that contains lower Mg concentrations (aka troughs). Given that *Clathromorphum* grow in temperate to arctic regions, SST is often correlated to the Mg/Ca cycles, signifying annual bands. Measured temperature during the microcosm experiment in Panama ranged from 24 °C to 29 °C with 90% of the experiment ranging only between 28 °C and 29 °C (Fig. 5). The peaks and troughs of the Mg/Ca and P/Ca cycles measured in this study show the same relationship to the dark, dense and lighter, less dense parts of the bands. The peaks align with the light-colored sections, while the troughs align with the dark-colored sections. Since we know from the experiment duration marked by staining that these

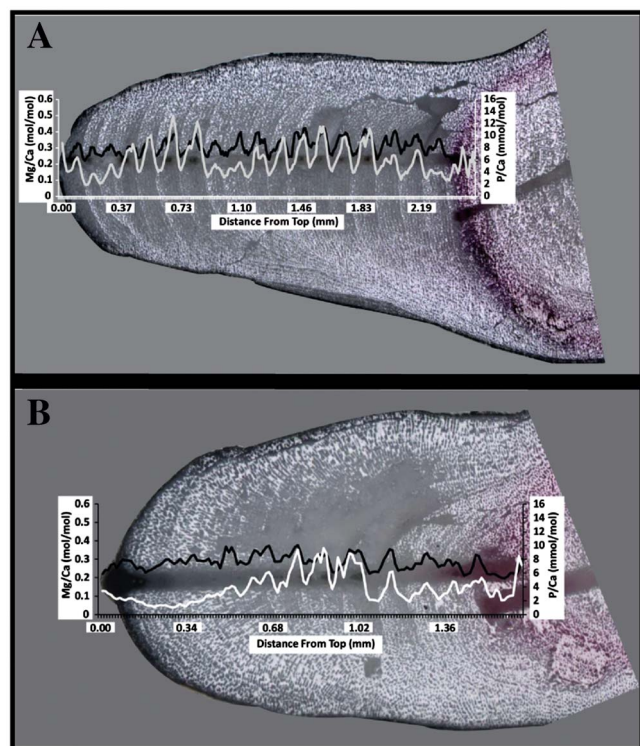


Fig. 3. Examples of Mg/Ca and P/Ca alignment with growth band cyclicality in post-ARS growth (i.e. experimental growth for 172-days). (A) Sample R5B (Axis B) has 18 bands and 18 elemental ratio cycles (e.g. strong correlation). (B) Sample R8B1 (Axis B) has 12 bands and 12 weaker elemental ratio cycles for Mg/Ca and about 9 cycles for P/Ca (e.g. moderate correlation). Black profile lines are Mg/Ca ratios and white profile lines are P/Ca ratios. Analytical error of laser data is within acceptable reproducibility using MACS-3 with %RSD (Mg is 3.5% and P is 11.8%).

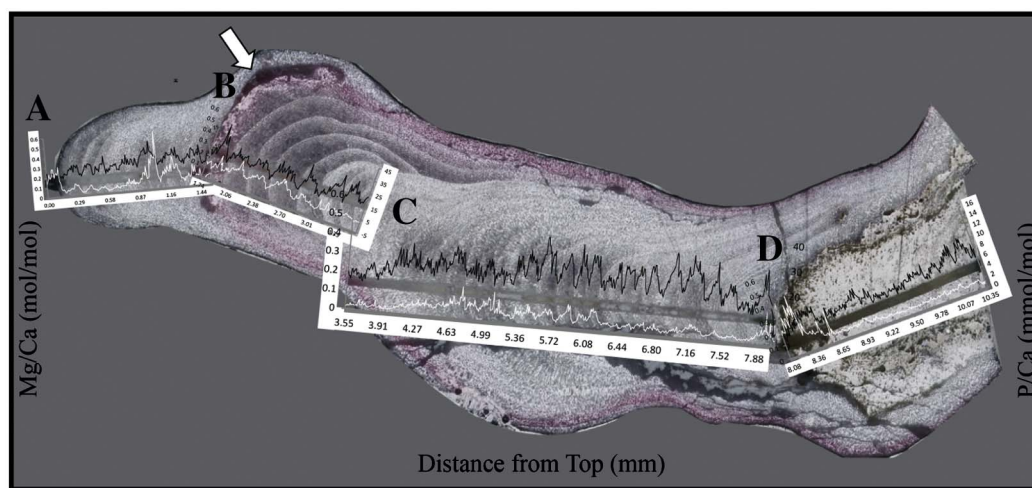


Fig. 4. Example of a rhodolith branch showing LA-ICP-MS laser transects (A–D) for both the pre- and post-ARS growth (sample R4A – from axis A). White arrow points to the outer layer of the pinkish-red ARS stain line marking the start of the experimental growth. The overlays are the Mg/Ca ratios (black lines) and the P/Ca ratios (white lines). All x-axes are measured best aligned distances of transect lines from the top of the branch. Transects B and C show P/Ca ranging on the right axis from 0 to 45 mmol/mol, while transects A and D range from 0 to 16 mmol/mol. All four transects for the Mg/Ca ratios range on the left axis from 0 to 0.6 mol/mol. Note that transect D was run through a core section of the branch that contained a highly diagenetically altered piece of rhodolith branch marked by a chalky white composition and no visible banding or cell delination. Analytical error of laser data is within acceptable reproducibility using MACS-3 with %RSD (Mg is 3.5% and P is 11.8%). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

are sub-annual bands, the Mg/Ca and P/Ca cycles cannot be annual, and do not appear to be controlled by temperature. Instead, growth rate (aka skeletal extension as indicated by growth bands) appears to exhibit stronger controls on the incorporation of Mg and P in the calcitic skeletal structure of these algae.

3.2. Influence of calcification versus extension rates on elemental ratios

Growth rates can be measured in two ways: (1) skeletal extension rate (mm yr^{-1}) and (2) calcification rate ($\text{mg mm}^{-2} \text{yr}^{-1}$) (Gillikin et al., 2005). Considering that the initial experimental results show a significant relationship between the total amount of new, post-ARS growth and skeletal extension rate ($R^2 = 1$; $p\text{-value} \leq 0.0001$; $n = 20$; see Sletten et al., 2017), here we see a moderately correlated, significant linear relationship between annual growth rate and the average Mg/Ca ($R^2 = 0.41$; $p\text{-value} = 0.007$; $n = 16$) as well as with P/Ca ratios ($R^2 = 0.38$; $p\text{-value} = 0.010$; $n = 16$) of the post-ARS growth (Fig. 6). When these relationships are restricted to the 8 samples that showed a strong relationship to the number of bands, these values increase to higher values for both Mg/Ca ($R^2 = 0.67$; $p\text{-value} = 0.013$; $n = 8$) and P/Ca ($R^2 = 0.64$; $p\text{-value} = 0.018$; $n = 8$) (Fig. 6). These significant observations between average elemental ratios and annual growth rates further support that the samples with longer, more continuous growth provide data demonstrating a relationship between skeletal extension and elemental incorporation. Weaker correlations derived from the total sample group ($n = 16$) may be due to growth during short time intervals being compared at an annual timescale.

The range in average Mg/Ca is between 0.20 and 0.31 ± 0.03 mol/mol and the overall average is 0.28 mol/mol for all branches ($n = 16$). These ratios are within or near the range of those documented for other CA that are assumed to grow annual increments such as New Caladonia *Sporolithon durum* Mg/Ca (0.19–0.49 mol/mol; Darrenougue et al., 2014), Greenland *Lithothamnion glaciale* (~ 0.09 –0.17 mol/mol; Kamenos et al., 2008, 2012), and GOP *Lithothamnion* sp. (0.0–0.34 mol/mol; Schäfer et al., 2011). The average P/Ca ratios range from 1.6 to 8.7 ± 2.15 mmol/mol with an overall average of 4.1 mmol/mol. Since there are no published data for P/Ca for CA we compare these values to the P/Ca measured in the coral species *Pavona gigantea* in the GOP (mean P/Ca = 0.12 mmol/mol). The offset is likely

due to the difference between coral and algal calcification as well as differences in P/Ca partition coefficients between calcite and aragonite (Ishikawa and Ichikuni, 1981; LaVigne et al., 2008).

If skeletal extension rate cannot fully explain the cyclicity of elemental content in the rhodoliths, perhaps the calcification rate addresses this discrepancy. Calcification rate accounts for the amount, or density, of HMC generated in a given space over time compared to extension rate, which simply is a measure of lateral carbonate extension over time. Calcification rate is ultimately driven by calcification processes (e.g. photosynthesis and [metabolic] diffusion), which have been connected to an “organic matrix-mediated process” that regulates the rate of heterogeneous elemental nucleation within the cell wall (Borowitzka and Larkum, 1987). Because of photosynthesis, calcification rate is light stimulated (Borowitzka and Larkum, 1987), and thus dependent on light exposure. As was found in the growth experiment performed by Sletten et al. (2017) rhodoliths in the GOP respond the most to light exposure, and appear to be less sensitive to temperature, salinity, or pH given that these parameters were all equal amongst the samples throughout the experiment. If overall algal growth is dependent mainly on light, then calcification rate may play a more significant role than previously considered and account for more of the variance than the skeletal extension can explain alone. In GOP *Lithothamnion* sp. this appears to happen without a significant delay between uptake and precipitation of CaCO_3 given that the cycles of Mg/Ca and P/Ca correlate directly to the number of growth bands in the post-ARS growth for at least 56% of the sample group. Since the measurement of calcification rate was not within the scope of this project, further study of rhodolith densities is required to assess the relationship between trace elemental content, band frequency, and calcification rate.

3.3. P/Ca_{alga} relationship to SRP and Mg/Ca_{alga} relationship to Mg/Ca_{sw}

There was a non-significant relationship between the average P/Ca_{alga} and SRP ($R^2 = 0.002$; $p\text{-value} = 0.61$; $n = 130$; Fig. 7). The correlation between Mg/Ca_{alga} and Mg/Ca_{sw} was slightly negative ($R^2 = 0.04$; $p\text{-value} = 0.03$; $n = 130$; Fig. 7). Low significance may in part result from the averaging method (see methods), which assumed constant growth of the rhodoliths given that the only time constraints to mark growth were the ARS line at the start of the experiment, and the edge of the branch sample marking the end of the experiment.

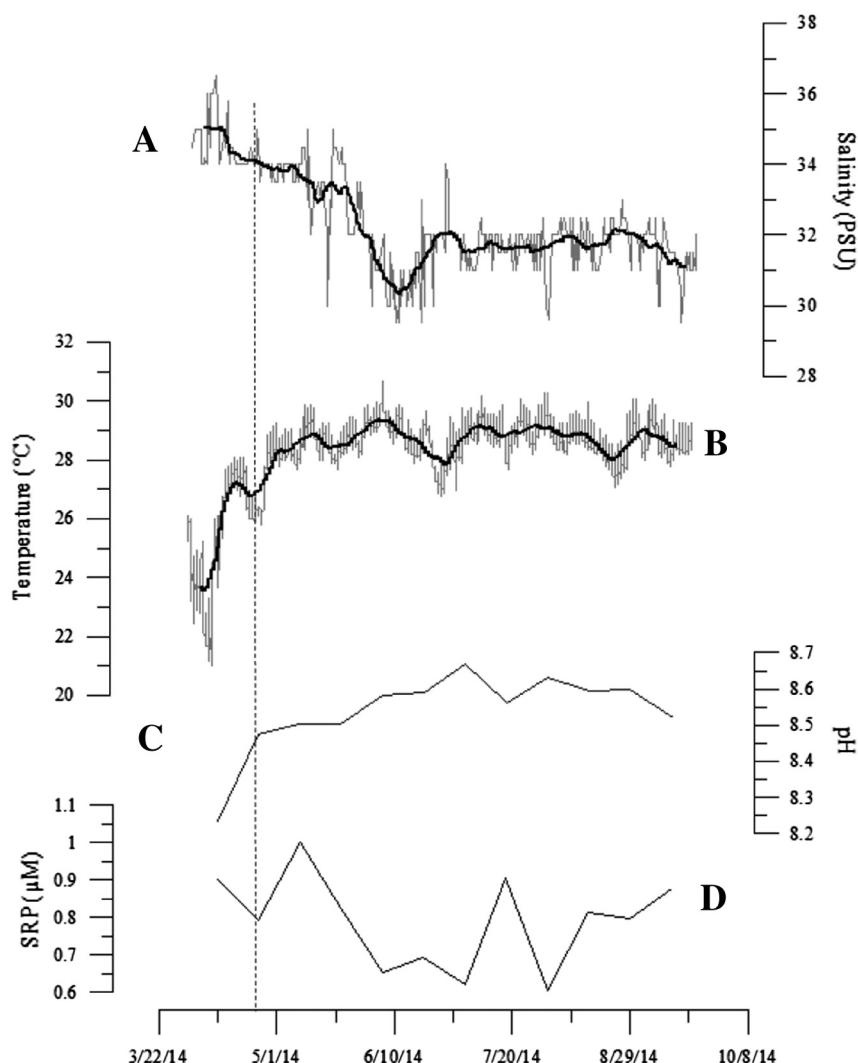


Fig. 5. Water conditions during the experiment (March 27 – September 19, 2014). Parameter A (salinity) shown with a 10-day moving average for daily measurements. Parameter B (temperature) shown with a 5-day moving average for hourly measurements. Parameters C (pH) and D (SRP [soluble reactive phosphorus]) were collected as individual samples on a bi-monthly basis. The dashed line marks the tail-end of the upwelling season. SRP was measured to collect a range of concentrations over the duration of the experiment.

Averaging the elemental data also may present mismatches to the SRP data since the elemental data has a higher resolution compared to the seawater chemistry and thus the elemental uptake during that period of growth between seawater sample events is likely not perfectly aligned. However, the averaging does characterize the growth through time, again assuming constant growth. The other reason that the P/Ca relationship to the seawater phosphate did not appear significant may be that the rhodoliths do not uptake dissolved inorganic phases of P (i.e. SRP also known as orthophosphate), and that instead the rhodoliths use predominantly dissolved organic phases (e.g. various P-esters) or organic condensed phosphates (e.g. ATP) for growth (Aydin et al., 2009; Jarvie et al., 2002). These latter phases were not measured as part of this study. If future work identifies similar weak relationships as found here between P/Ca and SRP, then the *Lithothamnion* sp. from the GOP are not a viable proxy for seawater phosphate, despite their ability to record P/Ca cycles. This would differ from LaVigne et al. (2010) who observed a strong linear relationship between P/Ca_{coral} and SRP for both coral species they worked with ($r^2_{\text{Porites lutea}} = 0.93$; $r^2_{\text{Montastrea sp.}} = 0.91$). However, they do leave their discussion open to other species of P having an influence on skeletal P/Ca and indicate the need for further proxy development using contemporaneous SRP data.

Mg/Ca_{sw} during the experiment ranged from 5.4 to 5.9 mol/L, which is within the normal range of Mg/Ca for seawater (e.g. Ries, 2006). This small range is unlikely to influence the patterns of Mg/

Ca_{alga}, particularly in conjunction with sub-annual band cyclicality. More extreme variation in the Mg/Ca_{sw} is needed to explain significant oscillations in Mg/Ca_{alga} as was demonstrated by Ries (2006) who used Mg/Ca_{sw} between 1.0 and 5.2 mol/L to alter algal growth and elemental uptake.

3.4. Biological and upwelling influences on Mg/Ca and P/Ca incorporation in *Lithothamnion* sp. in the GOP

It is possible that the dissimilarity in elemental content in different genera or species of the CA can also be explained by biological differences, as has been seen in other biogenic carbonates such as ostracods (Decrouy et al., 2012) or bivalves (Gillikin et al., 2005). One species of CA may internally regulate elemental use and concentrations based simply on physiological needs (i.e. vital effects). Alternatively, the cause or mechanism by which a certain species grows incremental growth bands may influence elemental ratios and their correlation to parameters like SST. The rhodolith former *L. glaciale*, for instance, has been shown to grow annual growth bands (Kamenos et al., 2008, 2012). However, these bands are largely driven by SST, such that the Mg/Ca cycles align with SST because SST fluctuates on a seasonal basis in these locations. It may be that species that grow at latitudes where solar insolation drives seasonality produce elemental sinusoidal patterns that represent the annual shift in temperature because the algae grow first-

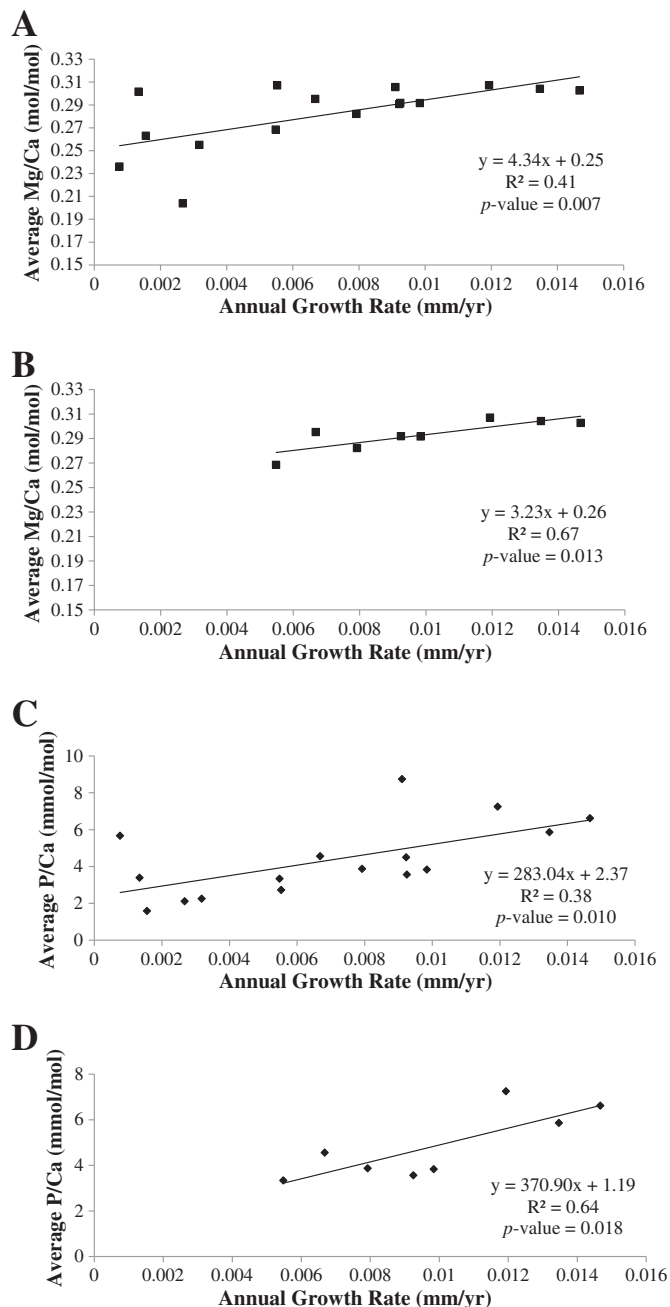


Fig. 6. (A) and (C) Data represent all 16 branch samples analyzed for LA-ICP-MS in this experiment. (B) and (D) show data for the 8 branch samples that display a strong correlation between the number of Mg/Ca or P/Ca cycles to the number of bands in the post-ARS growth.

tier bands in annual increments. This would support why this annual relationship is not seen in the tropical GOP rhodoliths since the tropics are not affected by winter and summer insolation.

Compared to higher-latitude CA that grow during the summer season and reduce or stop their growth in the winter, *Lithothamnion* sp. in the GOP appear to grow multiple bands year-round (either continuously or non-continuously) despite the upwelling conditions that generate a SST swing of $\sim 15^\circ\text{C}$ each year. Sub-annual banding is supported by the algae recording cyclic P through the duration of the experiment in the non-upwelling season instead of only peaking at a shift between upwelling and non-welling seasons. This is also despite the fact that this nutrient becomes a limiting nutrient during the non-upwelling season. In arctic regions, these nutrients (e.g. P and N) are limiting because they typically are not present at a high enough

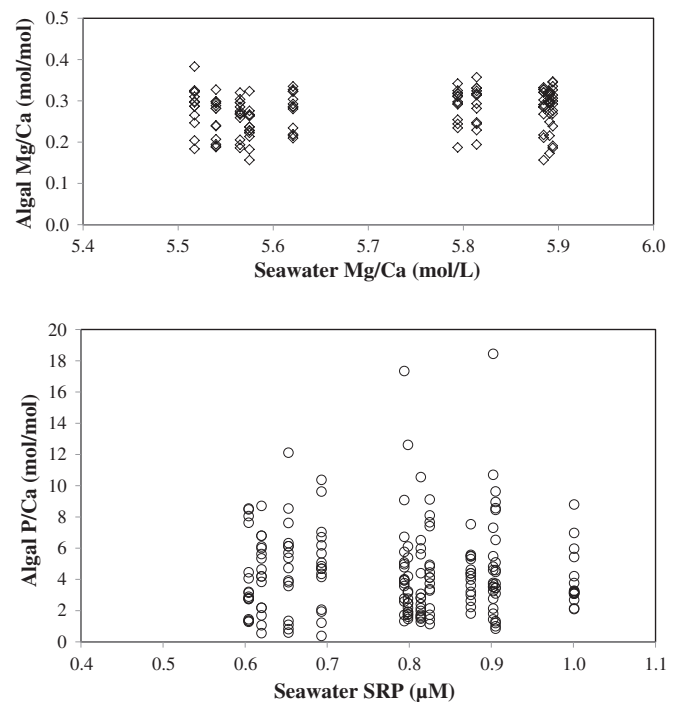


Fig. 7. Non-significant relationships between $\text{Mg}/\text{Ca}_{\text{alga}}$ and $\text{Mg}/\text{Ca}_{\text{sw}}$ ($R^2 = 0.03$) and the $\text{P}/\text{Ca}_{\text{alga}}$ and SRP (soluble reactive phosphorus) ($R^2 = 0.0002$). Samples ($n = 16$). Samples to measure $\text{Mg}/\text{Ca}_{\text{sw}}$ were collected 10 times during the experiment. To compare the $\text{Mg}/\text{Ca}_{\text{alga}}$ data to the seawater data, $\text{Mg}/\text{Ca}_{\text{alga}}$ values were equally divided into 10 groups and averaged. The same was done for $\text{P}/\text{Ca}_{\text{alga}}$ and SRP except that the $\text{P}/\text{Ca}_{\text{alga}}$ data were grouped into 12 groups and averaged because there were 12 SRP sample events. This comparison method assumes constant growth.

concentrations in the water column to stimulate constant bioproductivity (Freiwald and Henrich, 1994; Lüning et al., 1990). It would be expected then that P is more abundant during the cold, upwelling season in the GOP due to re-suspension of nutrients from deeper waters. During the experiment, SRP ranged between 0.6 and $1.0 \pm 0.12 \mu\text{M}$ accounting for a $\sim 40\%$ oscillation. Comparatively, $\text{P}/\text{Ca}_{\text{alga}}$ measured across the sample group varied by $\sim 98\%$. This is > 2 times the variability seen in the SRP. Thus, growth or perhaps vital effects are changing the elemental concentration in the algal skeleton that consequently produces elemental cycles with measurable amplitude. These biological controls may account for some of the unexplained relationships to extensional growth rate. Furthermore, the P/Ca ratio profiles closely match those of the Mg/Ca suggesting that the P could be recorded in situ with the Mg in the calcite crystal structure, or that both are incorporated into the organic material as has been seen with trace elements in other biogenic carbonates such as bivalves (Takesue et al., 2008). For example, the Mg content in the bivalve *Arctica islandica* can be significantly increased when measured in situ by LA-ICP-MS due to the inclusion of the bivalve's insoluble organic matrix (IOM). Using analogous samples without the IOM, the Mg content was also measured using ICP-OES (optical emission spectrometry) and found to be lower in concentration (Schöne et al., 2010). Although the growth and calcification of bivalves is different from CA, further investigation is suggested to separate the organic matter from the CA calcite skeleton using oxidative cleaning methods and measure the amount of Mg/Ca and P/Ca left in the calcite.

LaVigne et al. (2008, 2010) used Sr/Ca ratios of the coral (*Porites lutea* and *Montastrea* sp.) to determine annual SST that reflected upwelling conditions. The P/Ca measured in the coral correlated with the Sr/Ca, which led them to conclude that the P/Ca recorded seawater phosphate variation. Although their work showed strong correlation between P/Ca and SRP using best-fit regressions, y-intercept offsets in the calibration alluded to biologically-induced “vital effect” influences

on growth rates and P uptake into the carbonate skeleton of the coral. Here, we potentially see a similar impact on both the Mg/Ca and P/Ca ratios of the GOP rhodoliths.

4. Conclusions

The following conclusions can be made from this study:

- (1) The number of Mg/Ca and P/Ca ratio cycles strongly align with the number of growth bands accreted in the experimental growth in 50% of the samples from 9 rhodoliths from the GOP.
- (2) The Mg/Ca cycles observed in this study do not correlate to SST and do not show annual cycles given that the cycles align with multiple bands formed within a 172-day growth experiment.
- (3) Moderate linear correlation and statistically significant relationships are shown between Mg/Ca and annual growth rates, as well as P/Ca and annual growth rates.
- (4) Light is the main control on skeletal extension, suggesting that light indirectly influences the elemental uptake of Mg and P into the crystal lattice of *Lithothamnion* sp. from the GOP.

These results have implications for researchers attempting to use rhodoliths for SST and other paleoenvironmental reconstructions using trace elemental content, as well as using the Mg/Ca cycles as markers for annual growth bands.

Acknowledgements

We would like to thank Carlos Guerra and all of the STRI staff who helped make this work possible in Panama. Special thanks to Ansley Griffith, Collin Williams, and Christine Bassett with their assistance in sample preparation at The University of Alabama. We also thank Yuehan Lu and Peng Shang at the Organic Geochemistry Laboratory at The University of Alabama for assistance with nutrient data analysis. Funding was in part provided by a Smithsonian Tropical Research Institute Graduate Research Fellowship. We also thank the U.S. National Science Foundation for funding Union College's Perkin Elmer ICP-MS (NSF-CCLI #9952410) and CETAC LSX-213 laser ablation system (NSF-MRI #1039832).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.chemgeo.2017.05.010>.

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