Microstructural variation in oxygen isotopes and elemental calcium ratios in the coral skeleton of *Orbicella annularis*

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Abstract

Corals have long been used as proxies for changes in the sea-water environment. However, the existence of trace element and isotopic inconsistencies between different coral species and within different architectural elements of the same species grown simultaneously suggests that factors besides seawater temperature and its chemical composition contribute to coral geochemical records. Secondary Ion Mass Spectrometry (SIMS) analysis was conducted on the reef building coral *Orbicella annularis* from the Veracruz Reef System, in the Southern Gulf of Mexico in order to investigate variability in oxygen isotopes ($\delta^{18}\text{O}$) and elemental calcium ratios between the different architectural structures of this coral. The microstructures of *Orbicella* include thecae-walls, septa, costae, exothecal dissepiments (ExD), and endothecal dissepiments (EnD). SIMS data showed that EnD is isotopically heavier by 0.7 ‰ than other simultaneously grown structures. The elemental heterogeneity between architectural structures was observed by LA-

ICP-MS: EnD was found to become depleted in Mg/Ca, B/Ca and enriched in Ba/Ca, U/Ca relative to thecae. Elemental and isotopic spot profiles (with a 6.5 mm long sampling path) in the coral growth direction yielded weekly time resolution and demonstrated that factors other than temperature — including aragonite growth rate and modification of calcifying fluid chemistry — also affect elemental and isotopic ratios in corallite aragonite.

Keywords: Coral reefs, microscale calcification, oxygen isotopes, elemental ratios, SIMS, LA-ICP-MS

Introduction

Our understanding of climate change and oceanographic variability through time is largely derived from knowledge of oxygen isotopes ($\delta^{18}\text{O}$) (e.g. Fairbanks, 1979; Weil et al., 1981; Cole and Fairbanks, 1990; Leder et al., 1996; Wellington et al., 1996). Ratios of trace elements compared to calcium in coral skeletons have also been explored as potential paleothermometers (e.g. Weber, 1973; Smith et al., 1979; Beck et al., 1997; Wei et al., 2000; Cardinal et al., 2001; Watanabe et al., 2001; Quinn and Sampson, 2002; Cohen et al., 2004; Allison and Finch, 2007). However, the use of coral chemistry as a proxy for temperature has been complicated by inconsistencies between different temperature calibrations and temperature-independent heterogeneities of individual specimens (e.g. Land et al., 1975; Leder et al., 1996; Watanabe et al., 2002; Corrège, 2006; Giry et al., 2010; Gagan et al., 2012; DeLong et al., 2013; Sadler et al., 2014).

The complex microstructure of *Orbicella annularis* (previously known as *Montastraea annularis*) is formed by a few morphologically different units (architectural structures): thecae

46 wall, septa, costae, exothecal dissepiments (ExD), and endothecal dissepiments (EnD) (Figures
47 1-3). Detailed descriptions of the coral architecture are presented in Dávalos-Dehullu et al.
48 (2008). The thecae are the vertical walls in which the coral polyps live. The septa are the
49 vertical structures located between thecae walls. Costae are the bumps on the coral surface
50 surrounding the entrance to the thecae where the polyp extrudes (i.e. extensions of septa outside
51 of the thecae). The exothecal dissepiments (ExD) are horizontal plates that connect one thecal
52 wall to the thecal wall of an adjacent coral polyp. Endothecal dissepiments (EnD) are horizontal
53 plates the polyp builds under itself. In addition to differences in the position and orientation, it
54 was suggested for *Orbicella* species that the formation of dissepiments is linked to lunar cycles
55 (Dávalos-Dehullu et al., 2008).

56 Several studies have demonstrated isotopic heterogeneity in simultaneously-grown
57 aragonite from corallites. Leder et al. (1996) reported a stronger $\delta^{18}\text{O}$ response to temperature in
58 thecae than in endothecal regions, which represents the mixture of different architectural
59 structures of *O. annularis*. This temperature dependence caused a number of studies to conduct
60 sampling along the thecal wall to avoid geochemical signals from endothecal regions (Swart et
61 al., 2002; Smith et al., 2006; DeLong et al., 2011; Flannery and Poore, 2013). In most studies the
62 difference between ExD and EnD was not specified. For example, Watanabe et al. (2002)
63 observed differences in $\delta^{18}\text{O}$ between vertical (thecae) and horizontal (dissepiments) structures in
64 *Orbicella faveolata* and concluded that thecae is the most suitable structure for
65 paleothermometry. Because the spatial resolution of micromilling techniques usually exceed 50
66 μm , their $\delta^{18}\text{O}$ data for dissepiments likely represent the mixture of ExD and EnD (or ExD only)
67 and are therefore insufficient to sample EnD whose widths vary from 20 to 40 μm (Dávalos-
68 Dehullu et al. 2008). Our work provides higher spatial resolution for $\delta^{18}\text{O}$ and trace element data

in individual architectural structures of *O. annularis*. The data were obtained with Secondary Ion Mass Spectrometry (SIMS) and Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) with analytical spot sizes of 20-30 μm .

Coral sampling and analytical methods

Sample description and preparation

The coral species *Orbicella annularis* was collected at 10 m depth on the leeward slope of Isla Verde Reef (96° 04' W and 19° 12' N), Veracruz Reef System, Southern Gulf of Mexico, in August 1991. Pieces 1-2 cm long were sliced from the youngest side of the coral (Figure 1a). Specimens were sliced parallel and perpendicular to the direction of coral growth. This sample preparation technique means the parallel-sliced section exposed a time series of a continuously extended skeleton (Figure 1b) whereas the perpendicular-sliced section exposes near-simultaneously grown skeletal material (Figure 1c). The extension rates of the thecae and other skeleton structures of the coral were not directly measured in this study. We assumed the growth rates were similar to the rates estimated for the same coral species in the same area documented by Carricart-Ganivet (2004). No tissue layers were removed from the sample, but SIMS measurements confirmed a lack of organic material in the area analyzed (see details below). A section of continuously extended skeleton was examined for oxygen isotopes and elemental ratios from the coral surface to 6.5 mm depth. This corresponds to a growth period of 0.65 to 1.63 years using an extension rate range of 4-11 mm/yr of *Orbicella annularis* (Carricart-Ganivet, 2004; Dávalos-Dehullu et al., 2008). Both sections were mounted in epoxy (EpoxiCure®, Buehler) and polished with SiC papers (400, 800, and 1200 grit) and diamond

suspensions of 3 and 1 μ m sizes. The section perpendicular to the coral growth direction was taken at 5 mm from the surface of the coral (Figure 2). Thecae, septa, and costae are exposed in this section. It is reasonable to assume that these corallite structures precipitated simultaneously at near-constant temperature and seawater composition. The section parallel to the coral growth direction consists of the last 6.5 mm of growth prior to coral collection and shows corallite aragonite crystallized under variable temperature due to seasonal change (Figure 3a). Red boxes correspond to the areas at 2 ± 0.4 and 6 ± 0.1 mm from coral surface. Corallite depth in *O. annularis* is around ~ 4 mm (Dávalos-Dehullu et al., 2008), which is sufficient spatial offset between EnD and the other architectural elements. Therefore, it was assumed that EnD at 6 mm away from the surface grew simultaneously with thecae, septa, and ExD located at 2 mm from the coral surface (Figure 3). Magnified images at the right show LA-ICP-MS ablation pits in EnD (Figure 3b) and thecae with ExD (Figure 3c). In figures 3a,b the numbers of LA-ICP-MS spots are lower than the numbers of SIMS spots in the rectangle marked areas in figure 3a.

Micro-sampling was utilized to compare IRMS $\delta^{18}\text{O}$ data with $\delta^{18}\text{O}$ measured by SIMS in order to correct for SIMS instrumental mass fractionation. Three samples of aragonite were micromilled from the same coral slab used for SIMS and LA-ICP-MS analyses. Micro-sampling was conducted in the direction of coral growth 5 mm away from the parallel SIMS analytical profile (Figure 3a). The diameter of the drilled holes was 350 μm , locations of the sampled areas were 1.7, 3.8, and 6.1 mm from the coral surface. Collected aragonite powders were analyzed for $\delta^{18}\text{O}$ together with CaCO_3 SIMS reference calcite (MEX) and aragonite (BB-Ar) using an automated carbonate preparation device (KIEL-III) combined with the isotope ratio mass spectrometer (IRMS) Finnigan MAT 252 in the Environmental Isotope Laboratory at the

University of Arizona. Powdered samples were reacted with dehydrated phosphoric acid under vacuum at 70°C. The isotope ratio measurement was calibrated based on repeated measurements of NBS-19 ($\delta^{18}\text{O}_{\text{VPDB}}=-2.20$ ‰) and NBS-18 ($\delta^{18}\text{O}_{\text{VPDB}}=-23.2$) with a precision of ± 0.1 ‰ (1σ). Calibration on VPDB scale was based on the principle of identical treatment of standards and unknowns, thus the $\delta^{18}\text{O}$ value of CO_2 derived from NBS-19 and NBS-18 formed the basis of the VPDB scale for all unknowns.

Geochemical analysis

SIMS: Oxygen isotopes

SIMS $\delta^{18}\text{O}$ analyses were conducted along the simultaneously-deposited growth architectural structures and across transects in the directions parallel and perpendicular to coral growth using the CAMECA ims-1270 ion microprobe at the University of California Los Angeles. Samples were examined with a 2-3 nA Cs^+ primary beam with a lateral dimension of 20-30 μm at the sample surface (see Gabitov et al., 2012 for details). Negative secondary ions of ^{16}O and ^{18}O were simultaneously measured by Faraday Cups (FC) using multi-collection set-up for a mass resolving power (MRP) of ~ 2000 , which was sufficient for resolving hydride interference with the ^{18}O peak (Fayek et al., 2002). The raw intensity of ^{18}O in the standard and corals varied from $6.7 \cdot 10^6$ to $7.1 \cdot 10^6$ counts per second (cps). The total sputtering time prior to acquisition was 120 s, which was sufficient to reach a steady $^{18}\text{O}/^{16}\text{O}$ value over the 12 cycles of analysis on a single spot. A natural clear-beige aragonite crystal (BB-Ar) from Belice, Bohemia was used to normalize coral data. Ten spot analyses of BB-Ar yielded a standard deviation (s.d.) of 0.25 ‰. The standard was analyzed routinely after every 5-8 measurements of the coral. In

order to quantify SIMS data, intensity ratios of $^{18}\text{O}/^{16}\text{O}$ were standardized internally to the reference $^{18}\text{O}/^{16}\text{O}$ obtained from the bulk IRMS analyses (see Appendix-1 for details). The oxygen isotope analyses were performed in two analytical sessions. In the 1st analytical session EnD was only exposed near the edge of the round mount (>6000 μm from the center). According to zircon analysis by Kita et al. (2009), measurements this far from the center of the mount can introduce additional 0.3-0.4 ‰ fractionation in $\delta^{18}\text{O}$ relative to the center of the sample. Our isotopic profiles from -5000 to +5000 μm yield $\delta^{18}\text{O}$ standard deviations less than 0.25 ‰, whereas fractionations greater than 0.25 ‰ were observed outside of this region, likely due to the position-sensitive instrumental fractionations described by Kita et al. (2009). To reduce this instrumental fractionation, before the 2nd analytical session the coral sample was removed from the epoxy mount using a circular diamond saw and remounted in order to place EnD within +/- 5000 μm from the center. Further samples were polished identically to the 1st session prior to $\delta^{18}\text{O}$ and elemental ratios analyses.

SIMS: Li/Ca, B/Ca, Mg/Ca, and Sr/Ca ratios in 6.5 mm thecae wall

SIMS trace element analysis was conducted via transects in the direction parallel to the coral growth on spots adjacent to $\delta^{18}\text{O}$ measurements. Samples were analyzed with a 1.8-7.7 nA $^{16}\text{O}^-$ primary beam at 20-30 μm lateral dimension of the sample surface. Positive secondary ions corresponding to mass/charge stations of 6.5 (background), ^7Li , ^{11}B , ^{24}Mg , ^{42}Ca , and ^{88}Sr were measured in monocollection mode. Intensities were measured by peak switching with waiting times up to four seconds and counting times of 2, 2, 1, 1, and 2 seconds for ^7Li , ^{11}B , ^{24}Mg , ^{42}Ca , and ^{88}Sr , respectively. Each spot was pre-sputtered for 2 minutes. A mass resolving power (MRP) of ~2900 was sufficient to achieve separation of molecular interferences such as $^6\text{Li}^1\text{H}$, $^{10}\text{B}^1\text{H}$, $^{23}\text{Na}^1\text{H}$, $^{26}\text{Mg}^{16}\text{O}$, $^{40}\text{Ca}^1\text{H}_2$ (Gabitov et al., 2011). To reduce $^{44}\text{Ca}_2$ and $^{87}\text{Sr}^1\text{H}$

interferences ^{88}Sr was measured with the offset of -80 V using the energy bandwidth of 50 V (total voltage was 10 kV). Various CaCO_3 reference materials were used to determined molar element to calcium ratios: NBS-19 ($\text{Mg}/\text{Ca}=17.717$ mmol/mol, $\text{Sr}/\text{Ca}=0.191$ mmol/mol) (Gabitov et al., 2013); M93-TB-FC-1 (*Porites* coral with $\text{B}/\text{Ca}=0.364$ mmol/mol) (calculated from Kasemann et al., 2009); CAL-HTP (synthetic calcite where $\text{Li}/\text{Ca}=28.9$ $\mu\text{mol}/\text{mol}$) (calculated from Rollion-Bard et al., 2009). Count rates of ^{42}Ca varied from $2.1 \cdot 10^5$ to $2.3 \cdot 10^5$ cps in both the aragonite standard and coral sample indicating the absence of a significant amount of tissue material in the analyzed spots.

LA-ICP-MS: B/Ca, Mg/Ca, Sr/Ca, Ba/Ca, and U/Ca ratios in thecae, ExD, and EnD

Additional elemental analysis was performed for the different architectural structures of the coral using Laser Ablation Inductively Coupled Plasma Mass Spectrometer (LA-ICP-MS) at Rensselaer Polytechnic Institute. The LA-ICP-MS system consists of: a detachable Photon Machines Analyte 193 nm ultra-short pulse excimer laser ablation workstation and a Bruker 820-MS quadrupole inductively coupled plasma mass spectrometer (ICP-MS). ^{11}B , ^{24}Mg , ^{44}Ca , ^{87}Sr , ^{138}Ba , ^{238}U were analyzed using ^{43}Ca as an internal standard. The laser-spot was a rectangular slit 30×30 μm pulsing at 6 Hz with an $8.2 \text{ J}/\text{cm}^2$ laser energy. Helium flow was 0.63 liters per minute. Data reduction was performed using the Iolite software program (Paton et al., 2011). Mg/Ca and Sr/Ca were determined using reference calcite from UC Irvine UCI-CC ($\text{Mg}/\text{Ca}=3.446$ mmol/mol, $\text{Sr}/\text{Ca}=0.2043$ mmol/mol) (Gabitov et al., 2013). Reference calcite LAS-20 ($\text{U}/\text{Ca}=0.1193$) was used to evaluate U/Ca (Sano et al., 2005). B/Ca was verified using silicate glass standard NIST SRM 612 ($\text{B}/\text{Ca}=1.510$ mmol/mol calculated from Pearce et al.,

1997; Table 1 overall average, Table 9 preferred average). OKA carbonatite calcite was used for evaluation of Ba/Ca (Ba/Ca=1.61 mmol/mol, Gaetani and Cohen, 2006).

Results

IRMS analyses yielded $\delta^{18}\text{O}$ values varying from -4.77 to -3.84 ‰ in the different zones of the coral thecae, with the observed range corresponding to different calcification episodes of the coral growth. The observed isotopic range of 0.93 ‰ yields temperature change of 4°C using previously reported data (Grossman and Ku, 1986; Kim et al., 2007; Watanabe et al., 2001). This is within the range of the averaged monthly temperatures for the coral growth period obtained from Hadley Center (satellite SST for Veracruz), where temperature varied from 23.65°C in February 1990 to 29.76°C in August 1991.

SIMS micro-analyses of the perpendicular section of *O. annularis* show small isotopic heterogeneities between septa and costae (Figures 2 and 4a). Multiple (n=8 and 9) SIMS measurements yielded a standard error (s.e.=s.d./ $\sqrt{n}$) lower than 0.16 ‰, which allows resolving 0.44 ‰ differences between $\delta^{18}\text{O}_{\text{septa}}$ (-4.91 ‰) and $\delta^{18}\text{O}_{\text{costae}}$ (-5.35 ‰) (Table 1). The value of $\delta^{18}\text{O}_{\text{thecae}}$ overlaps with $\delta^{18}\text{O}_{\text{septa}}$ and $\delta^{18}\text{O}_{\text{costae}}$ within standard error. Sections parallel to the direction of coral growth exposed thecae, septa, ExD, and EnD structures, which presumably grew simultaneously (Figure 3 and 4b). Of the structures exposed in the growth-parallel sections, EnD is the most isotopically heavy structure where $\delta^{18}\text{O}_{\text{EnD}}$ =-3.19 ‰, which is higher than $\delta^{18}\text{O}$ of thecae and ExD by 0.7 ‰. A Student's t-test between thecae (n=9) and EnD (n=11) $\delta^{18}\text{O}$ values yielded a probability (p) of $3.2 \cdot 10^{-4}$, highlighting the significance of heavy isotope enrichment in EnD. Two $\delta^{18}\text{O}_{\text{septa}}$ values overlap with the mean of eleven EnD measurements

suggesting the enrichment of the heavy isotope in the polyp-hosting structures. Figure 5 shows variability of $\delta^{18}\text{O}_{\text{thecae}}$ at the different growth episodes of the 6.5 mm coral fragment measured by SIMS and three micromilled data points (large empty circles). SIMS measurements were conducted with large 500 μm steps and smaller 50-100 μm steps. The 50-100 μm steps correspond to the smallest growth episodes of 1.83-4.6 days, assuming a range of coral extension rate from 4-11 mm/yr (Carricart-Ganivet, 2004; Dávalos-Dehullu et al., 2008). It was observed that EnD is always isotopically heavier than thecae.

SIMS analyses of Li/Ca, B/Ca, Mg/Ca, Sr/Ca along thecal oxygen isotope transect did not yield strong correlation with each other or with $\delta^{18}\text{O}_{\text{thecae}}$ (Table A-1), although we observed a significant positive Pearson correlation with Mg/Ca ($r=0.43$; $p < 0.05$) and negative with Sr/Ca ($r=-0.44$; $p < 0.05$). The most significant association observed was the colinearity between Sr/Ca and Mg/Ca, where a linear fit yielded the following relationship: $\text{Sr/Ca} = -0.27 \cdot \text{Mg/Ca} + 10.5$ (mmol/mol); $r^2=0.43$; $p < 10^{-4}$. The other trends include: 1) $\delta^{18}\text{O}_{\text{thecae}}$ and B/Ca positive correlation with Sr/Ca, where $r^2 < 0.22$ and $p < 0.02$; 2) positive correlation of Mg/Ca with Li/Ca, where $r^2=0.19$ and $p=0.0047$.

LA-ICP-MS B/Ca, Mg/Ca, Sr/Ca, Ba/Ca, and U/Ca collected in thecae match the ratios determined by SIMS at similar distances from the coral surface (Table A-1). Also, LA-ICP-MS analyses were conducted in the thecae and ExD at a distance of 2 mm from the surface of the coral. The same elemental ratios were measured in EnD located at 6 mm from the coral surface (i.e. 2 mm from the polyp base) in order to compare geochemistry of architectural structures presumed to grow simultaneously. Table 2 and Figure 6 contain averaged elemental ratios of thecae, ExD, and EnD structures. According to this information, Mg/Ca is reduced by 16 % in EnD relative to thecae and ExD. In contrast Ba/Ca and U/Ca are greater by 56 and 15 %, respectively.

respectively, in EnD. Sr/Ca is also enriched in EnD relative to thecae and ExD by 1.7 % and 4.8 %, respectively. Both dissepiments have similar B/Ca ratios, which are 7.4 % lower than thecal B/Ca.

Discussion

The most significant isotopic heterogeneity in *O. annularis* was observed between EnD and thecae. $\delta^{18}\text{O}_{\text{EnD}}$ is consistently higher than any of the measured $\delta^{18}\text{O}_{\text{thecae}}$. This isotopic difference suggests instrumental fractionation due to the spatial offset between EnD and thecae in the epoxy mount from the first analytical session cannot explain ^{18}O enrichment in EnD. No analytical artifact can explain this variability, as $\delta^{18}\text{O}_{\text{thecae}}$ values of the same region were similar in both sessions. Our data are consistent with previous studies in that the isotopic composition of thecae is different from $\delta^{18}\text{O}$ of the mixed dissepiments (Land et al., 1975) and various architectural structures by ~ 1 ‰ (Leder et al., 1996; Watanabe et al., 2002). However, ^{18}O fractionation between thecae and other structures could vary regionally and seasonally (Leder et al., 1996; Watanabe et al., 2002). Here we compared $\delta^{18}\text{O}$ of the structures for one coral region in a single specimen.

The coral fragment shown in Figure 3a is 6.5 mm in length. Considering a range of extension rates (4-11 mm/yr), this corresponds to 0.59-1.63 years of growth. The SIMS analytical profile through the thecae did not reflect a clear winter-summer seasonal pattern associated with temperature, which precludes accurate temperature evaluation from corallite $\delta^{18}\text{O}$ through the growth interval of this sample. Our SIMS $\delta^{18}\text{O}$ profiles with 1.8-18 day temporal resolution yielded isotopic signals not entirely consistent with expected $\delta^{18}\text{O}$ records

controlled by seasonal SST change (Figure 5). According to the temperature records (Hadley Center), near maximum SST values (near minimum $\delta^{18}\text{O}$ values) are expected to be recorded by the coral edge which grew immediately prior to sample collection in August 1991. The thecal $\delta^{18}\text{O}$ is expected to increase moving away from coral edge and reach its maximum in thecae grown 6 month prior collection (i.e. February 1991). Therefore, seasonal temperature change is not the only factor affecting isotopic records in the studied sample. We could not evaluate the input of seasonal change in $\delta^{18}\text{O}_{\text{seawater}}$ (and hence on $\delta^{18}\text{O}_{\text{coral}}$) because no salinity data were available for the growth period of the coral fragment.

Several studies have reported an influence of riverine freshwater input on $\delta^{18}\text{O}_{\text{seawater}}$ and $\delta^{18}\text{O}$ of corals located close to the coastline (e.g. Gagan et al., 1998; Marshall and McCulloch, 2002; Sadler et al., 2014). These studies showed no significant effect of fresh water flooding on Sr/Ca. Furthermore, fresh water input should elevate Li and B content and decrease $\delta^{18}\text{O}$ and Mg in the coastal zone. We did not observe the consistent abrupt changes in $\delta^{18}\text{O}$ and element Ca ratios that would indicate freshwater input. This suggest a negligible effect of fresh water flux on coral geochemistry in the sample collection area between 1990 and 1991.

For the first time, our study documents for *O. annularis* that U/Ca and $\delta^{18}\text{O}$ are enriched in EnD relative to thecae— contrary to B/Ca. This observation suggests that fluid composition at the site of calcification changes significantly during growth of EnD and thecae. In other words, EnD grows from an environment with lower CO_3^{2-} and pH (i.e. lower calcification rate of EnD) relative to thecae (assuming a similar response of coral geochemistry to change of CO_3^{2-} in seawater and at the site of calcification). It was observed that U/Ca in deep-sea corals and synthetic aragonite increases with decreasing CO_3^{2-} or pH of seawater (Anagnostou et al., 2011; Raddatz et al., 2014; DeCarlo et al., 2015). B/Ca is predicted to decrease with decreasing pH as

the fraction of $\text{B}(\text{OH})_4^-$ (the aqueous species preferentially incorporated into aragonite) is pH sensitive (e.g. Palmer et al., 1987; Dickson, 1990; Hemming and Hanson, 1992; Sen et al., 1994). Based on our data and relationships between boron and pH the potential pH difference in dissepiments and thecae does not exceed 0.1 (Hemming and Hanson, 1992; Allison and Finch, 2010). The high $\delta^{18}\text{O}_{\text{EnD}}$ values found are also consistent with depletion of CO_3^{2-} and lower pH relative to the thecal growth environment assuming that several dissolved carbonate species contribute to $\delta^{18}\text{O}$ in coral (Zebee, 1999). Here, involvement of HCO_3^- (which is isotopically heavier than CO_3^{2-}) in coral calcification could affect $\delta^{18}\text{O}$ of different architectural structures of *Orbicella annularis*. The decrease in pH at the EnD calcification site could potentially be explained by an increase in CO_2 flux to this area and higher local respiration rates. More intense proton exchange mechanisms (e.g., H^+ -ATPases), may also facilitate the local enrichment in H^+ concentration and the consequent reduction in pH.

An additional explanation for the observed trace element and isotopic heterogeneity is that EnD growth is slower than thecae (thecae extension rate is 4-11 mm/yr). Experiments on inorganically precipitated aragonite demonstrated decrease of ^{18}O fractionation factor between aragonite and fluid with increasing of aragonite growth rate (Gabitov, 2013). Using his relationship between oxygen isotope fractionation and aragonite growth rate over the range of 0.06 to 0.88 nm/s (i.e. 2-28 mm/yr), the observed $\delta^{18}\text{O}_{\text{EnD}} - \delta^{18}\text{O}_{\text{Thecae}} = 0.7\text{‰}$ suggests the EnD grow slower than thecae by a factor of 3 (Figure 4b). These non-uniform growth rates are consistent with low Mg/Ca in EnD relative to thecae and data on Mg partitioning between abiotic aragonite and fluid [where growth rate enhances Mg incorporation into aragonite (Gabitov et al., 2008)]. Mg/Ca in EnD is 10% lower than in thecae, suggesting EnD grows slower than thecae by the factor of 2-3 using relationships from Gabitov et al. (2008) (Figure 6). The extension rate

range of *Orbicella annularis* is 4-11 mm/yr, which yields an EnD extension rate of 1.3-3.7 mm/yr assuming that the thecae growth rate is similar to the whole coral extension rate and that EnD grows slower than thecae by the factor of 3. The growth rate of 0.5-1.3 mm/yr is calculated using EnD thickness (0.02-0.05 mm) assuming each EnD grows during the whole lunar cycle, and 26 EnD formed annually. The discrepancy between EnD growth rate range (1.3-3.7 mm/yr) derived from $\delta^{18}\text{O}$ and Mg/Ca data and the growth rate range (0.5-1.3 mm/yr) estimated from EnD thickness could be explained by underestimation of the relationship between EnD thickness and its growth time due to a lack of information for precise rates of EnD formation. Isotopic data for *Pavona clavus* confirm a negative relationship between $\delta^{18}\text{O}$ and coral extension rate for a growth rate range between 1 and 5 mm/year (McConnaughey 1989a; Felis et al. 2003). No literature data on the $\delta^{18}\text{O}$ – growth rate relationship were found for *Orbicella*. Decreases in pH and CO_3^{2-} should decrease the fluid saturation state with respect to aragonite ($\Omega_{\text{aragonite}}$), which will consequently decrease the coral calcification rate. Therefore, the local variation in all three parameters (CO_3^{2-} , pH, and calcification rate), are potentially responsible for the observed distinct geochemistry of EnD documented here for *O. annularis*.

Seasonal changes in coral physiology and in seawater chemistry at the collection area may also affect the local environment at the site of calcification, and hence the geochemistry of *Orbicella*, as it has been documented for $\delta^{18}\text{O}_{\text{thecae}} - \delta^{18}\text{O}_{\text{mix}}$ in Leder et al. (1996) and Watanabe et al. (2002). It is important to recognize that coral calcification is a biological process and thus mediated by enzymes and membrane exchange processes. This implies that the local conditions for coral calcification could be strongly regulated by cell activity (which can broadly change across coral tissues) near the mineralization process.

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Appendix 1. SIMS IMF for standards and sample

Four reference CaCO_3 materials (BB-Ar, NBS-18, NBS-19, and MEX) were analyzed to determine SIMS instrumental mass fractionation [$\text{IMF} = 10^3 \cdot \ln(^{18}\text{O}/^{16}\text{O}_{\text{SIMS}} / (^{18}\text{O}/^{16}\text{O}_{\text{reference}}))$]. The reference isotopic ratios for NBS-18 ($\delta^{18}\text{O}_{\text{PDB}} = -23\text{‰}$) and NBS-19 ($\delta^{18}\text{O}_{\text{PDB}} = -2.2\text{‰}$) were obtained from Friedman et al. (1982). MEX ($\delta^{18}\text{O}_{\text{PDB}} = -10.39\text{‰}$) and BB-Ar ($\delta^{18}\text{O}_{\text{PDB}} = -7.79\text{‰}$) were analyzed by IRMS at the University of Arizona. No difference in IMF between calcite and aragonite standards was observed in the first analytical session. IMF values for calcite standards ($\text{IMF}_{\text{calcite}}$) were consistent to each other within analytical uncertainty, but lower than IMF of aragonite ($\text{IMF}_{\text{BB-Ar}}$) in the second analytical session, i.e. $\text{IMF}_{\text{BB-Ar}} - \text{IMF}_{\text{calcite}} = 0.8\text{‰}$. IMF of coral sample ($\text{IMF}_{\text{coral}} = -1.18\text{‰}$ and -2.55‰ for analytical sessions 1 and 2) was determined using isotopic ratios determined by SIMS and by IRMS. The two $^{18}\text{O}/^{16}\text{O}$ ratios for both methods

(SIMS and IRMS) were measured in thecae at the similar distances from the coral surface.

IMF_{coral} was used to correct the $\delta^{18}\text{O}$ coral data determined by SIMS, i.e. $\delta^{18}\text{O}_{\text{corrected}} = \delta^{18}\text{O}_{\text{SIMS}} -$

IMF_{coral}. Here $\delta^{18}\text{O}_{\text{SIMS}}$ is the raw $\delta^{18}\text{O}$ of coral normalized to the $\delta^{18}\text{O}_{\text{VPDB}}$ in BB-Ar:

$$\delta^{18}\text{O}_{\text{SIMS}} = \left[\frac{(^{18}\text{O}/^{16}\text{O})_{\text{raw}}^{\text{sample}} / (^{18}\text{O}/^{16}\text{O})_{\text{raw}}^{\text{BB-Ar}}}{(^{18}\text{O}/^{16}\text{O})_{\text{VPDB}}} - 1 \right] \cdot 10^3$$

The reason for different SIMS instrumental fractionation between standard and sample is probably the polycrystalline nature of corallite aragonite, which yields the different ionization efficiency relative to monocrystalline standard (BB-Ar).

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 556

Figure-1

Figure-2

Coral slice perpendicular to growth direction

Figure-3

Figure-4

Figure5

Figure-6

Table 1. Averaged SIMS values of $\delta^{18}\text{O}$ in five architectural structures in *Orbicella annularis*

	n	$\delta^{18}\text{O}$	s.e.	n	$\delta^{18}\text{O}$	s.e.	n	$\delta^{18}\text{O}$	s.e.
		Perpendicular, session-1			Parallel, session-1			Parallel, session-2	
theca	8	-3.57	0.16	2	-3.79	0.145	11	-3.88	0.135
septa	9	-3.39	0.11	2	-3.13	0.155			
costa	8	-3.83	0.12						
ExD				2	-3.79	0.175			
EnD							13	-3.19	0.083

SIMS data collected on the sections oriented parallel and perpendicular to coral growth direction in the session 1 and 2. Standard error (s.e.) is equal to $\text{s.d.}/\sqrt{n}$

Table 2. Averaged LA-ICP-MS values of element to calcium ratios in three architectural structures in *Orbicella annularis*

	n	B/Ca	s.e.	Mg/Ca	s.e.	Sr/Ca	s.e.	Ba/Ca	s.e.	U/Ca	s.e.
theca	5	0.450	0.0062	4.657	0.0559	8.911	0.0721	8.413	0.5356	0.640	0.030
ExD	4	0.413	0.0150	4.819	0.1135	8.649	0.0778	7.898	0.1142	0.633	0.027
EnD	6	0.420	0.0132	3.977	0.1856	9.062	0.0410	12.749	0.8665	0.729	0.040

LA-ICP-MS data collected on the section oriented parallel to coral growth direction. B/Ca, Mg/Ca, Sr/Ca, are in mmol/mol; Ba/Ca and U/Ca are in $\mu\text{mol/mol}$. Standard error (s.e.) is equal to $\text{s.d.}/\sqrt{n}$

Figure 1. Preparation of coral specimen.

The most recently grown 6.5 mm fragment of coral was detached from a larger slice (a). The magnified view of the studied coral fragment oriented in parallel direction of the coral growth (b). Coral fragment oriented perpendicularly to the growth direction (c).

Figure 2. Micrographs of the polished section perpendicular to coral growth.

Section was sliced at the distance of 5 mm from the coral surface. Black ovals are the burn marks from SIMS $\delta^{18}\text{O}$ analysis.

Figure 3. Micrographs of the polished section parallel to coral growth direction.

The whole section shown where the “surface” of the coral corresponds to the latest growth layer (0 mm distance) (a). Here the burn marks (oval and rounded spots) are from SIMS isotopic and elemental analysis. Red boxes mark the areas of simultaneously grown thecae, septa, ExD and EnD, which geochemistry were compared. Magnified images show simultaneously grown theca and ExD (b) and EnD (c) with the burn marks from LA-ICP-MS analysis. Circle contours show the areas of micromilling with the sample number (1-3). The 3rd sample was collected at 6.8 mm from the coral surface.

Figure 4. Isotopic composition of different architectural structures.

The sections are perpendicular (a, SIMS analytical session-1) and parallel (b, SIMS sessions 1 and 2) to the coral growth. Error bars are the standard errors of the mean. Analytical precision for individual spots is 0.25 ‰.

Figure 5. Temporal isotopic transect in the direction of coral growth.

The most recent theca is at 0 mm. Error bars are the standard errors (1σ) of the individual spot measurement.

Figure 6. Element:calcium ratios of different architectural structures.

Error bars are the standard errors of the mean.