Calcification rate and shell chemistry response of the planktic foraminifer *Orbulina universa* to changes in microenvironment seawater carbonate chemistry

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ABSTRACT

We use LA-ICP-MS depth profiling to explore the sensitivity of shell chemistry of the symbiotic planktic foraminifer *Orbulina universa* to diurnal changes in the holobiont physiology, over a wide range of seawater pH and DIC compositions. B/Ca and U/Ca vary in concert with diurnal Mg/Ca banding, forming compositional bands that are qualitatively consistent with physiological modification of seawater carbonate chemistry (pH, $[\text{B}(\text{OH})_4^-]$ / $[\text{HCO}_3^-]$ and $[\text{CO}_3^{2-}]$) within the foraminiferal microenvironment by the net effects of photosynthesis, respiration and calcification. The amplitude of B/Ca banding broadly conforms to banding predicted using the bulk-shell B/Ca sensitivity to the carbonate chemistry changes in the foraminiferal microenvironment. U/Ca banding tends to be greater than predicted using the published bulk-shell sensitivity of this proxy to carbonate chemistry. This either suggests that carbonate chemistry changes in the foraminiferal microenvironment are greater than predicted by modelling and/or the published bulk shell calibration does not accurately reflect the U/Ca sensitivity at the micro-scale. A fourfold increase in seawater DIC composition (1026 to 4019 $\mu\text{mol kg}^{-1}$) is associated with significant increases in Sr/Ca and Mg/Ca partitioning, and a decrease in Mn/Ca partitioning into shell calcite. The accompanying fourfold increase in calcite saturation produces only a twofold increase in

calcification rate (0.14 to 0.28 , $\pm 0.02 \mu\text{m hr}^{-1}$), suggesting that seawater carbonate chemistry exerts only a small effect on foraminiferal calcification rates, but does have a significant influence on trace element incorporation at both the inter-shell and bulk-shell scale.

1. INTRODUCTION

The trace element and stable isotope compositions of calcium carbonate precipitated by planktic foraminifera differ significantly from inorganic calcite compositions precipitated from seawater. This includes many of the major proxies used for seawater temperature and carbonate system reconstruction (Mg/Ca , B/Ca , $\delta^{11}\text{B}$, $\delta^{18}\text{O}$; see Allen et al., 2011, 2012; Bijma et al., 1999; Lea et al., 1999; McCrea, 1950; Morse and Bender, 1990; Sanyal et al., 1996, 2000; Uchikawa et al., 2015). The reasons for the differences between biologically precipitated and inorganic minerals remain poorly understood and are often simply attributed to unspecified biological “vital effects” (Urey et al., 1951; Weiner and Dove, 2003). The uncertain influence of vital effects on trace element incorporation into foraminifer shells remains a significant impediment to our ability to reconstruct past seawater conditions from geochemical proxies.

In planktic foraminifera, vital effects include physiological processes (e.g., photosynthesis, respiration and calcification) that modify seawater chemistry in the external boundary layer (microenvironment), and internal processes that regulate the precipitation rate, composition, polymorph type, topology and orientation of calcium carbonate (de Nooijer et al., 2014). Several studies have documented large diurnal carbonate chemistry variations within the microenvironment of symbiotic foraminifera (Jørgensen et al., 1985; Köhler-Rink and Kühl, 2005; Rink et al., 1998), which can be explained by changes in the balance between respiration and photosynthesis of the host foraminifer and algal symbionts (Wolf-Gladrow et al., 1999; Zeebe et al., 1999, 2003). Daytime photosynthetic activity of algal

symbionts consumes CO₂ and raises pH in the microenvironment, whereas nighttime respiration of the host foraminifer and symbionts releases CO₂ and lowers pH in the microenvironment. This drives a large diurnal change of almost one pH unit adjacent to the shell surface of *Orbulina universa* (Köhler-Rink and Kühl, 2005; Rink et al., 1998). Seawater in the microenvironment provides the source of ions for biomineralisation (de Nooijer et al., 2014), although the chemistry of the internal calcification environment is modified by physiochemical processes used to promote or regulate calcification (Bentov et al., 2009; de Nooijer et al., 2009, Glas et al., 2012; Zeebe and Sanyal 2002). The extent of the connection between the external microenvironment and internal calcification environment is unknown, although they are certainly linked, given the architecture of the calcification site (Fig 1).

The large diurnal changes in pH, DIC and other carbonate system parameters in the foraminiferal microenvironment (Köhler-Rink and Kühl, 2005; Rink et al., 1998; Wolf-Gladrow et al., 1999) have the potential to influence and modify the incorporation of pH- and carbonate-system sensitive trace elements such as B/Ca and U/Ca. The proportions of dissolved boron species, boric acid (B(OH)₃) and borate (B(OH)₄⁻), in seawater are governed by acid-base equilibria, whereby most boron exists in the form of B(OH)₃ at low pH, and B(OH)₄⁻ at high pH (Hershey et al., 1986). If only the charged B(OH)₄⁻ ion is thought to be included into foraminiferal calcite, as proposed by Hemming and Hanson, (1992), the B/Ca of foraminifer shells can be used to infer information about past ocean carbonate system chemistry. However, recent calcite precipitation experimental studies have shown the exclusive incorporation of B(OH)₄⁻ might not be the case (e.g., Uchikawa et al., 2015). Despite this, the B/Ca ratio in foraminifer shells can be empirically related to the product of seawater the [B(OH)₄⁻]/[HCO₃⁻] ratio and the effective exchange coefficient (Yu et al., 2007):

$$\text{B/Ca} = K_{\text{D\_B/Ca}}^* \cdot ([\text{B(OH)}_4^-]/[\text{HCO}_3^-]) \quad (1)$$

where the square brackets indicate seawater concentrations, and $K_{D\_B/Ca}^*$ is the apparent (stoichiometric) exchange coefficient. Experimental culture studies confirm that the B/Ca ratio in planktic foraminifer shells increases with seawater pH (Allen et al., 2011, 2012; Henehan et al., 2013), as high pH drives higher $[B(OH)_4^-]$ and lower $[HCO_3^-]$ in solution, increasing the $[B(OH)_4^-/HCO_3^-]$ ratio. Thus, while the precise mechanisms behind B uptake into foraminifera are uncertain, we may relate B/Ca in our cultured specimens to changes in microenvironment $[B(OH)_4^-/HCO_3^-]$ and pH.

The U/Ca composition of foraminiferal calcite is also sensitive to seawater carbonate chemistry, and shows a non-linear decrease with increasing $[CO_3^{2-}]$ in pH experiments (Russell et al., 2004). The incorporation of uranium into coral aragonite is suggested to occur as a uranyl carbonate complex, most likely the triscarbonato ion, $(UO_2(CO_3)_3^{4-})$, which is the dominant uranyl species over the range of modern seawater pH (see Djogić et al., 1986). The $[UO_2(CO_3)_3^{4-}]$ ion likely requires a coordination change to fit into the calcite lattice (Reeder et al., 2000):

This reaction has been used to describe the U/Ca composition of calcite as a function of the triscarbonate species and effective exchange coefficient (Russell et al., 2004):

$$U/Ca = K_{D\_U/Ca}^* \cdot [UO_2(CO_3)_3^{4-}] / ([Ca^{2+}] \cdot [CO_3^{2-}]^3) \quad (3)$$

As the carbonate ion concentration and seawater pH increase, the cubic nature of the carbonate ion term in the partition coefficient denominator drives an exponential decrease in the sensitivity of U/Ca with carbonate ion activity in seawater.

To explore the response of shell trace element chemistry to seawater DIC and pH, and the importance of physiologically controlled microenvironment effects, we conducted a small number of culture experiments on the symbiotic planktic foraminifer *Orbulina universa*. *Orbulina universa* is an ideal species for this purpose, as it calcifies continuously to produce

a simple spherical shell comprising sequential diurnal growth bands (Eggins et al., 2004; Spero et al., 2015). Here, we use a laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) depth profiling technique (Eggins et al., 2004) to measure banded variations in Mg/Ca, B/Ca and U/Ca through the shell. We interpret changes in the magnitude and phase of banding in these elements in context of a physiological diffusion-reaction model of the foraminifer-symbiont system, and estimate growth rates from the well-established regular pacing of Mg/Ca banding. The magnitude of B/Ca and U/Ca banding is compared with the modelled diurnal variations in pH, $[B(OH)_4^-/HCO_3^-]$ and $[CO_3^{2-}]$ at the shell surface, combined with published sensitivities of the B/Ca and U/Ca proxies (Allen et al., 2011; Russell et al., 2004) to determine whether microenvironment changes are sufficient to drive B/C and U/Ca banding in *O. universa* (Fig. 2).

2. METHODS

2.1. Foraminifer collection and culturing

Orbulina universa were cultured using established methods (e.g., Russell et al., 2004) during August 2013 at the Wrigley Institute for Environmental Studies (WIES), on Santa Catalina Island, California. Small juvenile foraminifers were either hand collected by SCUBA divers, or by plankton tow in the San Pedro Basin surface waters approximately 2km NNE of WIES. Individual foraminifers were identified to species level and their largest diameter was measured under an inverted microscope before being transferred into 120 mL soda-lime glass jars (Wheaton®). The jars were filled with experimental seawater and sealed with a snap cap lid lined with Parafilm™ M laboratory film to limit gas exchange with the laboratory atmosphere. Foraminifers collected by plankton tow were transferred into experimental seawater only after they had regrown their spines, and in two cases, this transfer followed initial formation of their final spherical chamber.

Culture jars containing foraminifers were placed in circulating water baths held at constant temperature, 21.92 ± 0.13 °C, monitored with HOBO TidbiT® loggers. A 12-12 hr light-dark cycle was imposed for the duration of the culture experiments using Osram Lumilux T5 skywhite high output bulbs to deliver a PAR irradiance level greater than or equal to 285 ± 23 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$), to ensure light saturation of symbiont photosynthesis (Rink et al., 1998). Light intensity was monitored every 7-10 days with a Biospherical Instruments QSP-2000 light meter. Foraminifers were fed a 1-day old brine shrimp (*Artemia* sp. nauplius, San Francisco Bay strain) every second day. Foraminifers typically underwent gametogenesis several days after forming their adult-stage spherical shell. Following gametogenesis, empty shells were rinsed in deionized water, air-dried and archived before being cleaned and analyzed at the ANU.

Seawater obtained from the foraminifer collection site was filtered (0.8 μm) prior to use in experiments, and its pH and DIC adjusted to produce high and low DIC and high and low pH compositions (Table 1). Artificial seawater with the observed salt composition of seawater from Millero (1974) was made containing no DIC, and mixed with ambient seawater to produce experimental seawater with low DIC concentration ($1026 \mu\text{mol kg}^{-1}$). Sodium bicarbonate was added to ambient seawater to produce experimental seawater with double the ambient DIC concentration ($4019 \mu\text{mol kg}^{-1}$). Seawater pH was titrated with 0.1M NaOH or 0.1M HCl to produce target pH values of 7.53, 7.96/7.97 and 8.44 (total scale). Uranium and boron were enriched in experimental seawaters to facilitate subsequent microanalysis of B/Ca and U/Ca in foraminifer shells. Enrichment was achieved by addition of a uranium chloride and boric acid stock solutions, to levels of 50x ambient seawater U and 5x ambient seawater B concentration, except for the low DIC seawater which was enriched to just below half this level.

The total alkalinity of experimental seawater was measured by Gran-titration using a Metrohm 809 open cell autotitrator that was standardized using Dickson certified reference seawater. Seawater pH was measured with a Metrohm pH electrode that had been calibrated to the NBS scale using low ionic strength pH buffers (pH 4.00, 7.00 and 10.00; Fisherbrand). The pH and alkalinity of experimental seawaters were measured at the beginning and end of all experiments to monitor the consistency of carbonate system chemistry during experiments. Calculation of the carbonate system equilibria, and conversion from pH_{NBS} to the pH_{TOTAL} scale, were performed using a modified version of the van Heuven et al. (2011) Matlab script (CO2SYS.m) that accommodates changes to seawater $[\text{B}]_{\text{T}}$ independent of salinity. Uranium speciation was calculated from $[\text{U}]_{\text{T}}$ using aqueous thermodynamic data from Djogić et al. (1986). Small samples of experimental seawater were filtered using 0.22 μm Millipore syringe filters, transferred to acid cleaned 15 mL plastic vials and acidified to pH ~4 with optima grade HNO_3 for later trace element analysis at the ANU.

2.2. Sample Preparation and Analysis

Seawater trace metal concentrations were measured using a Varian Vista Pro Axial ICP-AES and a Varian 820 Quadrupole ICP-MS at the Research School of Earth Sciences, the Australian National University (RSES, ANU; Supplementary material, Table S1). Seawater samples were diluted 10x with 2% HNO_3 for analysis by ICP-AES using a three-point external calibration curve, run at the beginning and end of each analytical session, and a standard-sample-standard bracketing protocol to correct for instrument drift. Seawater B/Ca, Mg/Ca, and Sr/Ca molar ratios were measured with a reproducibility of $\pm 1.25\%$, $\pm 0.13\%$, and $\pm 0.16\%$ (1σ) based on repeated analysis of the CASS-4 seawater reference material (NRC, Canada). We also measured seawater by ICP-MS to measure Mn/Ca and U/Ca, which were below or near the detection limits of ICP-AES, and to validate B/Ca, Mg/Ca and Sr/Ca measurements. For ICP-MS analysis seawater samples were diluted 100x with 2% HNO_3 .

Instrument drift was monitored and corrected using a combination of internal standards (^9Be , ^{45}Sc , ^{89}Y , ^{115}In , and ^{235}U) and measurement of a synthetic seawater standard every five samples. Seawater B/Ca, Mg/Ca, Mn/Ca, Sr/Ca, and U/Ca molar ratios were measured with a precision of $\pm 0.9\%$, $\pm 1.3\%$, $\pm 1.5\%$, $\pm 1.0\%$, and $\pm 2.1\%$ (1σ) respectively, based on repeat analyses of CASS-4 seawater. Accuracy relative to average seawater ratios for B/Ca, Mg/Ca, Sr/Ca and U/Ca is 1.0%, 1.5%, 3.7% and 14% and Mn/Ca relative to CASS-4 is 1.9%.

Orbulina universa shells were oxidatively cleaned to remove organic material prior to LA-ICP-MS analysis by cracking shells open with a scalpel blade, followed by immersion in a 50:50 solution of 30% hydrogen peroxide and 0.1M NaOH in a water bath at 55 ± 5 °C for ~10 minutes (Pak et al., 2004). The buffered solution was then removed and the shell repeatedly rinsed with ultra-pure milli-Q water before being mounted on carbon tape for analysis (Eggins et al., 2003, 2004). Trace element/Ca (TE/Ca) ratios were measured using a Varian 820 Quadrupole ICP-MS coupled to an ArF excimer laser ($\lambda = 193\text{nm}$, pulse width = 25 ns FWHM) via an ANU Helex laser ablation system at the RSES, ANU following Eggins et al. (2003, 2004; Supplementary material, Table S2). Laser ablation sampling was conducted using 70 μm diameter circular spots at a laser pulse rate of 3 Hz at a fluence of $\sim 2\text{ J/cm}^2$. A mixed He-Ar gas stream was used to transfer ablation products to the ICP-MS. A rapid peak hopping protocol, employing 10 to 30 ms dwell times per isotope depending on abundance, was applied to measure selected analyte isotopes (^{10}B , ^{11}B , ^{24}Mg , ^{25}Mg , ^{27}Al , ^{43}Ca , ^{44}Ca , ^{55}Mn , ^{87}Sr , ^{88}Sr , and ^{238}U) repeatedly every ~ 0.35 seconds for a duration of 30-90 seconds, depending on shell thickness. Three profiles were measured from inside to outside through each foraminifer shell.

The ICP-MS instrument response was calibrated using SRM NIST610 glass and corrected for variation in ablation yield and instrument drift by normalizing to the measured internal standard isotope (^{43}Ca and ^{44}Ca) intensities during each mass spectrometer cycle. An

in-house carbonate standard was measured periodically to monitor the consistency of carbonate ablation relative to the SRM NIST 610. LA-ICP-MS data reduction followed well established procedures for time-resolved analysis (Longerich et al., 1996), including the initial screening of mass-time spectra for outliers and subtracting mean background intensities (measured with laser off). The mean TE/Ca ratio obtained from each compositional profile was averaged to produce a bulk shell composition for each specimen. Multiple profiles obtained from each foraminifer shell were combined to produce an average profile by identifying and aligning common peaks using the Linage function in AnalySeries v1.2, following Allen et al. (2011). To isolate only the banding patterns in our data, a Butterworth bandpass Fourier filter (Butterworth, 1930) was applied to the LA-ICP-MS profiles to remove low frequency (period > 5 μm) trends and high frequency (period < 0.5 μm) noise using the scipy.signal python package (Jones et al., 2001). The long period patterns are the gradually increasing trends of Mg/Ca, B/Ca and U/Ca that occur through the test from the inside of the POS to the outside of the shell, and short period patterns are instrumental noise.

The time taken to measure each shell compositional profile was converted to shell wall thickness and depth relative to the internal shell surface, assuming each laser pulse removed a 0.1 μm thick layer of calcite (Eggins et al., 2003). Linear calcification rate can then be converted to rate with units of mass per unit surface area per unit time using the density of calcite.

3. RESULTS

3.1 Intra-shell trace element compositional variation

The diurnal phasing of the variation shown by different trace elements is determined by comparison to the diurnal pattern of Mg/Ca banding (Fig. 3), which is characterized by high Mg bands formed at night and low Mg bands during the day (Spero et al., 2015). Measured trace element/Ca ratio profiles through the final spherical chamber wall of *O. universa* shells all show significant compositional growth banding (Fig. 3-Fig. 5). In most cases, the diurnal Mg/Ca banding is accompanied by a trend of increasing Mg/Ca away from the primary organic sheet (POS) towards the outside of the shell (see Fig. 3).

The POS is located in a band of relatively low Mg/Ca near the inner shell surface and in some cases associated with an anomalous high U/Ca band (Eggins et al., 2004; Sadekov et al., 2005). The number of low and high Mg/Ca band pairs developed between the POS and external surface is consistent with the number of days each foraminifer lived in experimental culture after formation of its final spherical chamber, as recorded in daily culture logs.

Profiles of Mg/Ca, B/Ca and U/Ca with the associated noise and background trends removed with a Butterworth bandpass filter are plotted in Fig. 4. B/Ca banding is generally out of phase with Mg/Ca, and where present U/Ca banding is broadly in phase with Mg/Ca in most shells (Fig. 4). Given the diurnal nature of Mg/Ca (Spero et al., 2015), this suggests that high B/Ca bands precipitate during the day and low B/Ca bands precipitate during the night, and the converse applies to U/Ca. While the number of specimens considered here is small, we observe that B/Ca and U/Ca banding are most clearly developed in the low pH and low DIC experiments (Fig. 4 a-d). U/Ca banding in the high DIC experiments is less well developed and possibly absent (Fig. 4 e-f).

Mn/Ca banding is notable for occurring in shells grown in low DIC seawater, but is not obvious in other experiments (Fig. 5). Sr/Ca shows no evidence of diurnal banding, although significant variations do occur in many shells. In particular, Sr/Ca ratios tend to be lower

between the POS and inner shell surface (Fig. 5). Other notable features are the presence of relatively high Mn/Ca and Mg/Ca ratios at the inner shell surface (Fig. 5).

3.2 *TE/Ca variations with carbonate system variables*

The mean bulk shell compositions of each experimental group (Fig. 6) are calculated using the average profile compositions of each shell, excluding the contribution of calcite precipitated prior to the experiment. Our bulk shell composition estimates have significant uncertainties, due to the small number of shells analyzed in each experiment and the variable compositions between individual shells. Accordingly, we caution against using the results reported here for the purpose of proxy calibration and reconstruction of seawater carbonate system parameters.

Bulk shell Mg/Ca and Sr/Ca compositions correlate with DIC, with the increase in Sr/Ca from 1.19 ± 0.07 to 1.71 ± 0.02 mmol/mol being highly significant (Pearson R correlations: Sr/Ca – DIC, $R = 0.98$, $p = 0.02$, $n = 4$; Mg/Ca – DIC, $R = 0.99$, $p < 0.01$, $n = 4$).

The B/Ca of shells measured in this study is high compared to natural specimens because of the elevated $[B]_T$ in our experimental seawaters. To compare data between our experiments, the B/Ca of the low DIC specimens must be corrected for the two-fold lower $[B]_T$ than other treatments. This correction is made using the B/Ca- $[B(OH)_4^-]$ relation of Allen et al. (2011) for *O. universa* ($B/Ca = 1.4 * [B(OH)_4^-] - 39.5$). After correction, bulk B/Ca has a positive trend with seawater $[CO_3^{2-}]$ ($R = 0.74$) and a positive trend with seawater pH ($R = 0.90$), although the correlations are not significant at the 95% confidence level ($p = 0.26$ and 0.09 , respectively).

Measured shell U/Ca values in the low DIC experiment are corrected for the 3.1x lower seawater $[U]_T$ in this experiment, assuming a linear relationship between shell U/Ca and seawater $[U]_T$ (Russell et al., 1994). The resulting set of corrected shell U/Ca compositions

form a negative non-linear trend with $[\text{CO}_3^{2-}]$ ($R = 0.86$, $p = 0.14$, $n = 4$), although the correlation is not significant.

Shell Mn/Ca values are low ($<1 \mu\text{mol/mol}$), except in the low DIC experiment, which had 4.7x higher $[\text{Mn}]_T$ than the other experiments (Fig. 5 a-b). The measured shell Mn/Ca in this experiment was linearly corrected to a 4.7x lower Mn/Ca value assuming partitioning scales with $[\text{Mn}]_T$ concentration (Eggins, unpublished data). Strong negative relationships are observed between Mn/Ca and both DIC (Mn/Ca – DIC, $R = -0.96$, $p = 0.04$, $n = 4$).

3.3 Partition coefficient changes with calcification rate

Mean linear growth rates are determined by dividing the shell wall thickness (profile length) by the observed number of diurnal growth band pairs (each of which reflects 24 hrs of growth). These rate estimates increase in experimental order from low DIC < low pH < high pH < high DIC (Supplementary material, Table S2), but are notable for falling in a narrow range between 0.14 ± 0.02 and $0.28 \pm 0.02 \mu\text{m hr}^{-1}$, despite the large range of saturation state (Ω) across these experiments. Our linear calcification rates measured for *O. universa* correspond to values of $R(\log_{10} \text{Rate mol m}^{-2} \text{s}^{-1}) = -6.5$ and -6.1 . Although the calcification rate uncertainties are large due to the small number of individuals grown in each experiment, a general increase in calcification rate with increasing calcite saturation state is apparent.

Apparent partition coefficients (K_D 's) are calculated for Mg/Ca, Sr/Ca and Mn/Ca by dividing the respective shell TE/Ca by seawater TE/Ca ratios. Because B and U do not substitute for Ca^{2+} , shell B/Ca and U/Ca are divided by $([\text{B}(\text{OH})_4^-]/[\text{HCO}_3^-])$ and $[\text{UO}_2(\text{CO}_3)_3^{4-}]/([\text{Ca}^{2+}] \cdot [\text{CO}_3^{2-}]^3)$; Equations 1 and 3), from carbon and boron species concentrations calculated in CO2SYS, and for uranium species concentrations calculated from Djogić et al. (1986) for each experimental condition. There are significant increases in $K_{D\_Mg/Ca}$ and $K_{D\_Sr/Ca}$ with calcification rate (Fig. 7; $R = 0.95$, 0.95 , and $p = 0.05$, 0.05 ,

respectively, $n = 4$), and a significant decrease in $K_{D\_Mn/Ca}$, with calcification rate (Fig. 7; $R = -0.99$, and $p < 0.01$, $n = 4$). No consistent relationship is observed for either $K_{D\_B/Ca}$ or $K_{D\_U/Ca}$ with growth rate.

4. DISCUSSION

4.1 *Intra-shell composition response to microenvironmental carbonate system variations*

The B/Ca and U/Ca compositional banding we document in *O. universa* shells is qualitatively consistent with the expected variation of these geochemical proxies due to diurnal changes in the effects of net photosynthesis-respiration on pH, $[B(OH)_4^-/HCO_3^-]$ and $[CO_3^{2-}]$ within the microenvironment of *O. universa* (Fig. 2). The spatial coherence of high B/Ca bands with low Mg/Ca bands that are formed during the day (Spero et al., 2015) (Fig. 4), is consistent with the daytime photosynthetic drawdown of CO_2 and resulting elevation of pH and $[B(OH)_4^-/HCO_3^-]$ in the microenvironment of *O. universa*. Similarly, the synchronicity of high U/Ca bands with high Mg/Ca bands that are formed during the night, is consistent with an increase in net respired CO_2 at night and resulting decrease in microenvironmental pH and $[CO_3^{2-}]$. The phasing of high U/Ca bands with high Mg/Ca bands are clearest in the low DIC and low pH experiments (Fig. 4 a-d). This is consistent with the enhanced sensitivity of U/Ca at the lower concentrations of $[CO_3^{2-}]$ of these experiments, described by both theory (Equation 3) and the empirical bulk shell calibration of Russell et al. (2004). Conversely, the absence of strong U/Ca banding in specimens grown in high DIC and high pH experiments is likely the result of the lower sensitivity of U/Ca to $[CO_3^{2-}]$ at high concentrations of $[CO_3^{2-}]$ (Russell et al., 2004) driving any U/Ca variations below our analytical precision.

In an effort to quantify the extent of the microenvironmental influence on shell chemistry, we combine the proxy calibrations of Allen et al. (2011) and Russell et al. (2004)

with the diffusion-reaction model developed by Zeebe et al. (2003) to predict compositional changes in shell B/Ca and U/Ca arising from the carbonate chemistry variation over diurnal cycles. The diffusion-reaction model was run for both light and dark conditions, using the results for the different seawater chemistries of each experiment as a basis for comparing observed and predicted shell compositions and diurnal variation. The model calculates concentration profiles of the carbonate system parameters in the chemical microenvironment of the foraminifer using fixed rates of photosynthesis, respiration and calcification of respectively 10, 2 and 3 nmol C h⁻¹ in the light, and 0, 2 and 1 nmol C h⁻¹ in the dark (Lea et al., 1995; Rink et al., 1998; Zeebe et al., 2003). The carbonate chemistry calculated at the shell surface in the dark (night) and the light (day) is then used to estimate the diurnal changes in B/Ca and U/Ca compositions based on published sensitivities to seawater carbonate chemistry. The de-trended profiles are plotted against the calculated diurnal composition changes in Fig. 8.

The amplitude of observed B/Ca banding between the POS and outer surfaces of shells generally matches the predicted compositional variation. However, the measured U/Ca variation is generally much greater than the predicted amplitude of U/Ca banding (Fig. 8 b, d, f, h). Possible explanations for this could be a greater U/Ca -[CO₃²⁻] sensitivity than previously reported by bulk calibrations (equal to or greater than 3 times) or differences in the relative carbon fluxes used in the model of Zeebe et al. (2003) to describe foraminiferal physiology. Differences in the strength of photosynthesis and calcification might explain some of the differences in banding within and between individuals (e.g., Fig. 8 i - j). However, varying calcification, photosynthesis and respiration carbon fluxes in the model over a range that exceeds the observed carbon flux values cannot explain the variation measured in both B/Ca and U/Ca bands (Fig. 8 i - j). This suggests that disparities from modelled bands could be the result of limitations associated with bulk calibrations. This may

be explained by the fact that, bulk shell calibrations integrate day and night calcite, thereby varying the individual contributions of light-dependent physiological processes on the internal carbonate system of the foraminifer. Disparities from predictions could also arise from internal biomineralization processes that influence U/Ca and B/Ca differently. For example, if foraminifers employ either carbon concentrating or proton pumping mechanisms, the associated changes to either DIC and/or pH will affect U and B incorporation differently, through their individual sensitivities to DIC and pH.

The diffusion-reaction model results are influenced by how far the symbiont swarm extends from the surface of the shell. As noted in our culture logs, in culture experiments, the density and position of the symbiont halo can vary greatly between individual foraminifers and throughout their ontogeny. This could also vary systematically with different experimental conditions as the symbionts might adjust their position subject to both light and carbon availability. Despite these caveats, the model demonstrates that the geochemical banding observed within *O. universa* shells is broadly consistent with diurnal changes in microenvironment carbonate chemistry produced by photosynthesis, respiration and calcification.

This is the first study to evaluate B/Ca and U/Ca banding in symbiotic planktic foraminifers, and extends the observations on *O. universa* reported by Allen et al. (2011). Comparable B data have been reported by Branson et al. (2015) who, using a high-resolution scanning transmission x-ray microscope, observe B bands that peak just after Mg bands in the benthic foraminifer *Amphistegina lessonii*. Although this appears to conflict with our result, it is important to note that this species belongs to a different suborder than *O. universa*, and that Mg/Ca banding in *A. lessonii* has been linked to chamber addition rather than having a diurnal origin (Bentov and Erez, 2005; Erez, 2003). The phasing of B/Ca and Mg/Ca banding

in this species is therefore likely to be driven by a distinct mechanism rather than the diurnal processes active in *O. universa*.

In contrast to good agreement between modeled and observed B/Ca in foraminifer shells outside the POS, predicted B/Ca does not match what was observed between the POS and the inner shell surfaces (see Fig. 8 a, c, e, g). Anomalous high U/Ca values at the position of the POS are a prominent feature in many shells, particularly in the low pH and high DIC experiments (Fig. 3 c-d, e-f). The origin of these elevated U/Ca bands is unclear, and might reflect initial calcification of the spherical adult chamber of *O. universa* at significantly lower $[\text{CO}_3^{2-}]$ than during subsequent shell calcification. Alternatively, high uranium contents associated with the POS might be a product of uranium adsorption or accumulation at this organic layer, in a similar manner to the sequestration of uranium carbonate complexes onto cell surfaces and associated components in marine cyanobacteria (Acharya et al., 2009). This feature might also be at the limit of LA-ICP-MS depth resolution, and therefore is not fully resolved in all shells.

Several assumptions underpin our ability to predict diurnal changes in shell compositions using the diffusion-reaction model. Firstly, the empirical proxy relationships we apply to our data have been calibrated using bulk shell compositions and over a range of seawater compositions that are much narrower than our DIC experiments. Secondly, the rates of photosynthesis and respiration are constant in the model, although they have been observed to change daily with changes in symbiont productivity (Spero and Parker, 1985) and throughout the foraminifer's ontogeny as it grows and symbiont numbers increase (Fujiki et al. 2014; Spero and Parker, 1985; Takagi et al., 2016). This might explain why B/Ca and U/Ca banding overlies broader compositional trends that occur with shell thickening over multiple days of growth (Fig. 3).

4.2 Bulk-shell TE/Ca composition response to carbonate system variations

The bulk B/Ca and U/Ca systematics observed here are qualitatively consistent with those documented for *O. universa* cultures by Allen et al. (2011) and Russell et al. (2004) (Fig. 6). Shell B/Ca compositions show a positive correlation with $[B(OH)_4^-/HCO_3^-]$ as expected based on equation 1, albeit with a significant positive intercept (Fig. 9). The trend and intercept are broadly consistent with results from previous studies, after taking into account the $[B]_T$ difference between studies.

The lack of a Mg/Ca-pH relationship is notable, given previously reported $7 \pm 5\%$ Mg/Ca sensitivity to pH (Russell et al, 2004). The absence of this relationship may be attributable to the high variability of Mg/Ca between individual shells (Sadekov et al., 2008; Spero et al., 2015), combined with the limited specimen numbers in our study. The variability of TE/Ca ratios between individuals could be due to a range of factors, for example, organism size, growth rate and genetic factors (Boyle, 1995), as well as the size, growth rate and location of the symbiont population.

4.2 Calcification rate effects on trace element incorporation

The twofold increase of foraminifer calcification rate over the large experimental range of DIC and Ω is significantly lower than the precipitation rate increase predicted for inorganic calcite. Using calcite precipitation rate calculations of Butron and Walter (1987), increasing solution saturation state from 2 to 8 results in an increase of inorganic precipitation rate by 20 times, this is an order of magnitude larger than for *O. universa*. The twofold increase in calcification rate from the low DIC to high DIC experiments (low to high $[CO_3^{2-}]$) is broadly consistent with a twofold increase in shell thickness over a ten-fold increase of $[CO_3^{2-}]$ observed Bijma et al. (1999) and Russell et al. (2004), who attributed this observation to increased shell calcification rates. Our $\log_{10}$ Rate values are consistent with previous rate

estimates for other planktic foraminifers (i.e. $R = -6.6$ to -6.0) grown over a similar range of seawater Ω (see Fantle and Tipper, 2014, and calculated using calcium isotope data from Gussone et al., 2003 and Kısakürek et al., 2011).

A wealth of experimental data and an extensive theoretical basis underpin evidence for the effect of calcification rate on trace element incorporation into calcite (see DePaolo, 2011; Gabitov and Watson, 2006; Lorens, 1981; Nielsen et al., 2012; Tesoriero and Pankow, 1996). In general, in inorganic calcite, Sr and other incompatible trace elements ($K_D < 1$) exhibit larger partition coefficients with increasing calcite precipitation rate, whereas Mn and other compatible trace elements ($K_D > 1$) experience smaller partition coefficients with increased precipitation rate (Dromgoule & Walter, 1990; Lorens, 1981; Tang et al., 2008; Tesoriero & Pankow, 1996). At faster precipitation rates trace element behaviour tends toward kinetic fractionation, whereas at slower precipitation rates equilibrium partitioning dominates (DePaolo, 2011). The apparent sensitivity of bulk shell Mg/Ca, Sr/Ca and Mn/Ca compositions to seawater carbonate system parameters could reflect the effect of these parameters on calcification rate, with Sr/Ca and Mg/Ca increasing and Mn/Ca decreasing with increasing calcification rate (and Ω). These results lend support to the previous suggestion of increasing Sr/Ca ratios in planktic foraminifers with increasing calcification rate (Dueñas-Bohórquez et al., 2009; Kısakürek et al., 2008; Lea et al., 1999). Variation in calcification rates might also contribute to observed compositional differences between calcite precipitated on either side of the POS in individual shells. Very thin diurnal Mg/Ca bands on the inside of the POS indicate calcification proceeds about 5 times slower at the inner shell surface than the outer surface in *O. universa* (Spero et al., 2015). These slower calcification rates might explain the higher Mn/Ca compositions and tendency toward lower Sr/Ca compositions between the inner surface and the primary organic sheet (see Figs. 5 and 7). Whether differences in day and night calcification rates might also be sufficient to

modulate the amplitude of diurnal banding remains to be established. This will require future use of higher resolution analysis methods to constrain day and night calcification rates.

Calcification rates slower than $R = -6.0$ are within the range where kinetic effects influence Sr and Mn partitioning into calcite (DePaolo, 2011; Lorens, 1981; Tang et al., 2008; Tesoriero and Pankow, 1996). However, they fall below the rates where kinetic effects have been shown to strongly influence inorganic B partitioning into calcite (i.e. $R > -6$, Uchikawa et al., 2015). The narrow range of shell calcification rates exhibited by *O. universa* further suggests that calcification rate is unlikely to exert a large influence on bulk shell B/Ca compositions. We suggest that both B/Ca and U/Ca are much more sensitive to ambient seawater carbonate chemistry at the intra-shell scale than rate, given that *O. universa* and other planktic foraminifers appear to regulate their shell calcification rates within narrow limits.

5. CONCLUSIONS

It is important to understand how and why trace elements vary within foraminifer shells, in order to constrain “vital effects” on trace element to calcium ratio proxies recorded in foraminiferal calcite. In *O. universa* the B/Ca compositional banding is out-of-phase with diurnal Mg/Ca banding, and U/Ca banding, where present, is in-phase with Mg/Ca banding. B/Ca banding and in many cases U/Ca banding are qualitatively consistent with the respective proxy responses to diurnal changes in the carbonate system chemistry within the chemical microenvironment of the foraminifer, based on B/Ca and U/Ca relationships established using bulk analytical methods. These carbonate system changes result from the net effects of respiration, calcification, and the photosynthetic activity of symbionts. However, there is a greater variation of U/Ca, and of B/Ca (in the high and low DIC experiments), within individual shells than predicted by microenvironment modelling. It is

not clear whether this is due to limitations with the sensitivities of the bulk shell proxy calibrations used, to the sizes of the net carbon fluxes attributed to photosynthesis and respiration in the modeled microenvironment, or additional factors that are specific to the biomineralization process. Thus, our current understanding of diurnal microenvironmental variability is sufficient to explain the relative phasing, but not amplitude of Mg/Ca, B/Ca and U/Ca banding.

A quadrupling of DIC and calcite saturation state of seawater only results in a doubling of shell calcification rates. The small number of foraminifers cultured and analysed in this study reveal that DIC concentration and calcification rate have positive correlations with bulk shell Sr and Mg partitioning, and a negative correlation with bulk Mn partitioning. No significant calcification rate effect is observed for bulk shell B and U partitioning. This may be a consequence of the small (factor of two) range in calcification rate, and greater sensitivity of these elements to seawater pH and DIC.

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493 REFERENCES

- 494 Acharya, C., Joseph, D., & Apte, S. K. (2009). Uranium sequestration by a marine
 495 cyanobacterium, *Synechococcus elongatus* strain BDU/75042. *Bioresource*
 496 *Technology*, 100(7), 2176-2181. doi: <http://dx.doi.org/10.1016/j.biortech.2008.10.047>
- 497 Allen, K. A., Hönisch, B., Eggins, S. M., & Rosenthal, Y. (2012). Environmental controls on
 498 B/Ca in calcite tests of the tropical planktic foraminifer species *Globigerinoides ruber*
 499 and *Globigerinoides sacculifer*. *Earth and Planetary Science Letters*, 351–352, 270-
 500 280. doi: <http://dx.doi.org/10.1016/j.epsl.2012.07.004>
- 501 Allen, K. A., Hönisch, B., Eggins, S. M., Yu, J., Spero, H. J., & Elderfield, H. (2011).
 502 Controls on boron incorporation in cultured tests of the planktic foraminifer *Orbulina*
 503 *universa*. *Earth and Planetary Science Letters*, 309(3–4), 291-301. doi:
 504 <http://dx.doi.org/10.1016/j.epsl.2011.07.010>
- 505 Bentov, S., Brownlee, C., & Erez, J. (2009). The role of seawater endocytosis in the
 506 biomineralization process in calcareous foraminifera. *Proceedings of the National*
 507 *Academy of Sciences*, 106(51), 21500-21504. doi: 10.1073/pnas.0906636106
- 508 Bentov, S., & Erez, J. (2005). Novel observations on biomineralization processes in
 509 foraminifera and implications for Mg/Ca ratio in the shells. *Geology*, 33(11), 841-844.
 510 doi: 10.1130/g21800.1
- 511 Bijma, J., Spero, H. J., & Lea, D. W. (1999). Reassessing Foraminiferal Stable Isotope
 512 Geochemistry: Impact of the Oceanic Carbonate System (Experimental Results). In G.
 513 Fischer & G. Wefer (Eds.), *Use of Proxies in Paleoceanography* (pp. 489-512):
 514 Springer Berlin Heidelberg.
- 515 Boyle, E. A. (1995). Limits on benthic foraminiferal chemical analyses as precise measures
 516 of environmental properties. *The Journal of Foraminiferal Research*, 25(1), 4-13.
- 517 Branson, O., Kaczmarek, K., Redfern, S. A. T., Misra, S., Langer, G., Tyliczszak, T., . . .
 518 Elderfield, H. (2015). The coordination and distribution of B in foraminiferal calcite.
 519 *Earth and Planetary Science Letters*, 416, 67-72. doi:
 520 <http://dx.doi.org/10.1016/j.epsl.2015.02.006>
- 521 Burton, E. A., & Walter, L. M. (1987). Relative precipitation rates of aragonite and Mg
 522 calcite from seawater: Temperature or carbonate ion control? *Geology*, 15(2), 111-
 523 114. doi: 10.1130/0091-7613(1987)15<111:rproaa>2.0.co;2
- 524 Butterworth, S. (1930). On the theory of filter amplifiers. *Wireless Engineer*, 7(6), 536-541.
- 525 de Nooijer, L. J., Spero, H. J., Erez, J., Bijma, J., & Reichart, G. J. (2014). Biomineralization
 526 in perforate foraminifera. *Earth-Science Reviews*, 135, 48-58. doi:
 527 <http://dx.doi.org/10.1016/j.earscirev.2014.03.013>
- 528 de Nooijer, L. J., Toyofuku, T., & Kitazato, H. (2009). Foraminifera promote calcification by
 529 elevating their intracellular pH. *Proceedings of the National Academy of Sciences*,
 530 106(36), 15374-15378. doi: 10.1073/pnas.0904306106
- 531 DePaolo, D. J. (2011). Surface kinetic model for isotopic and trace element fractionation
 532 during precipitation of calcite from aqueous solutions. *Geochimica et Cosmochimica*
 533 *Acta*, 75(4), 1039-1056. doi: <http://dx.doi.org/10.1016/j.gca.2010.11.020>
- 534 Djogic, R., Sipos, L., & Branica, M. (1986). Characterization of uranium(VI) in seawater.
 535 *Journal Name: Limnol. Oceanogr.; (United States); Journal Volume: 31:5, Medium:*
 536 *X; Size: Pages: 1122-1131.*

- Dromgoole, E. L., & Walter, L. M. (1990). Iron and manganese incorporation into calcite: Effects of growth kinetics, temperature and solution chemistry. *Chemical Geology*, 81(4), 311-336. doi: [http://dx.doi.org/10.1016/0009-2541\(90\)90053-A](http://dx.doi.org/10.1016/0009-2541(90)90053-A)
- Dueñas-Bohórquez, A., da Rocha, R. E., Kuroyanagi, A., Bijma, J., & Reichart, G.-J. (2009). Effect of salinity and seawater calcite saturation state on Mg and Sr incorporation in cultured planktonic foraminifera. *Marine Micropaleontology*, 73(3-4), 178-189. doi: <http://dx.doi.org/10.1016/j.marmicro.2009.09.002>
- Eggins, S., De Deckker, P., & Marshall, J. (2003). Mg/Ca variation in planktonic foraminifera tests: implications for reconstructing palaeo-seawater temperature and habitat migration. *Earth and Planetary Science Letters*, 212(3-4), 291-306. doi: [http://dx.doi.org/10.1016/S0012-821X\(03\)00283-8](http://dx.doi.org/10.1016/S0012-821X(03)00283-8)
- Eggins, S. M., Sadekov, A., & De Deckker, P. (2004). Modulation and daily banding of Mg/Ca in *Orbulina universa* tests by symbiont photosynthesis and respiration: a complication for seawater thermometry? *Earth and Planetary Science Letters*, 225(3-4), 411-419. doi: <http://dx.doi.org/10.1016/j.epsl.2004.06.019>
- Erez, J. (2003). The Source of Ions for Biomineralization in Foraminifera and Their Implications for Paleooceanographic Proxies. *Reviews in Mineralogy and Geochemistry*, 54(1), 115-149. doi: 10.2113/0540115
- Fantle, M. S., & Tipper, E. T. (2014). Calcium isotopes in the global biogeochemical Ca cycle: Implications for development of a Ca isotope proxy. *Earth-Science Reviews*, 129, 148-177. doi: <http://dx.doi.org/10.1016/j.earscirev.2013.10.004>
- Fujiki, T., Takagi, H., Kimoto, K., Kurasawa, A., Yuasa, T., & Mino, Y. (2014). Assessment of algal photosynthesis in planktic foraminifers by fast repetition rate fluorometry. *Journal of Plankton Research*, 36(6), 1403-1407. doi: 10.1093/plankt/fbu083
- Gabitov, R. I., & Watson, E. B. (2006). Partitioning of strontium between calcite and fluid. *Geochemistry, Geophysics, Geosystems*, 7(11), n/a-n/a. doi: 10.1029/2005GC001216
- Glas, M. S., Langer, G., & Keul, N. (2012). Calcification acidifies the microenvironment of a benthic foraminifer (*Ammonia* sp.). *Journal of Experimental Marine Biology and Ecology*, 424-425, 53-58. doi: <http://dx.doi.org/10.1016/j.jembe.2012.05.006>
- Gussone, N., Eisenhauer, A., Heuser, A., Dietzel, M., Bock, B., Böhm, F., . . . Nägler, T. F. (2003). Model for kinetic effects on calcium isotope fractionation ($\delta^{44}\text{Ca}$) in inorganic aragonite and cultured planktonic foraminifera. *Geochimica et Cosmochimica Acta*, 67(7), 1375-1382. doi: [http://dx.doi.org/10.1016/S0016-7037\(02\)01296-6](http://dx.doi.org/10.1016/S0016-7037(02)01296-6)
- Hemming, N. G., & Hanson, G. N. (1992). Boron isotopic composition and concentration in modern marine carbonates. *Geochimica et Cosmochimica Acta*, 56(1), 537-543. doi: [http://dx.doi.org/10.1016/0016-7037\(92\)90151-8](http://dx.doi.org/10.1016/0016-7037(92)90151-8)
- Henehan, M. J., Rae, J. W. B., Foster, G. L., Erez, J., Prentice, K. C., Kucera, M., . . . Elliott, T. (2013). Calibration of the boron isotope proxy in the planktonic foraminifera *Globigerinoides ruber* for use in palaeo-CO₂ reconstruction. *Earth and Planetary Science Letters*, 364, 111-122. doi: <http://dx.doi.org/10.1016/j.epsl.2012.12.029>
- Hershey, J. P., Fernandez, M., Milne, P. J., & Millero, F. J. (1986). The ionization of boric acid in NaCl, Na□Ca□Cl and Na□Mg□Cl solutions at 25°C. *Geochimica et Cosmochimica Acta*, 50(1), 143-148. doi: [http://dx.doi.org/10.1016/0016-7037\(86\)90059-1](http://dx.doi.org/10.1016/0016-7037(86)90059-1)
- Jones, E., Oliphant, T., & Peterson, P. (2001). Open source scientific tools for Python: Scipy.
- Kaczmarek, K., Langer, G., Nehrke, G., Horn, I., Misra, S., Janse, M., & Bijma, J. (2015). Boron incorporation in the foraminifer *Amphistegina lessonii* under a decoupled carbonate chemistry. *Biogeosciences*, 12(6), 1753-1763. doi: 10.5194/bg-12-1753-2015

- Kısakürek, B., Eisenhauer, A., Böhm, F., Garbe-Schönberg, D., & Erez, J. (2008). Controls on shell Mg/Ca and Sr/Ca in cultured planktonic foraminifera, *Globigerinoides ruber* (white). *Earth and Planetary Science Letters*, 273(3–4), 260–269. doi: <http://dx.doi.org/10.1016/j.epsl.2008.06.026>
- Kısakürek, B., Eisenhauer, A., Böhm, F., Hathorne, E. C., & Erez, J. (2011). Controls on calcium isotope fractionation in cultured planktic foraminifera, *Globigerinoides ruber* and *Globigerinella siphonifera*. *Geochimica et Cosmochimica Acta*, 75(2), 427–443. doi: <http://dx.doi.org/10.1016/j.gca.2010.10.015>
- Köhler-Rink, S., & Kühl, M. (2005). The chemical microenvironment of the symbiotic planktonic foraminifer *Orbulina universa*. *Marine Biology Research*, 1(1), 68–78. doi: 10.1080/17451000510019015
- Lea, D. W., Martin, P. A., Chan, D. A., & Spero, H. J. (1995). Calcium uptake and calcification rate in the planktonic foraminifer *Orbulina universa*. *The Journal of Foraminiferal Research*, 25(1), 14–23. doi: 10.2113/gsjfr.25.1.14
- Lea, D. W., Mashiotto, T. A., & Spero, H. J. (1999). Controls on magnesium and strontium uptake in planktonic foraminifera determined by live culturing. *Geochimica et Cosmochimica Acta*, 63(16), 2369–2379. doi: [http://dx.doi.org/10.1016/S0016-7037\(99\)00197-0](http://dx.doi.org/10.1016/S0016-7037(99)00197-0)
- Longerich, H. P. (1996). Laser ablation inductively coupled plasma mass spectrometric transient signal data acquisition and analyte concentration calculation. *Journal of Analytical Atomic Spectrometry*, 11, 899–904.
- Lorens, R. B. (1981). Sr, Cd, Mn and Co distribution coefficients in calcite as a function of calcite precipitation rate. *Geochimica et Cosmochimica Acta*, 45(4), 553–561. doi: [http://dx.doi.org/10.1016/0016-7037\(81\)90188-5](http://dx.doi.org/10.1016/0016-7037(81)90188-5)
- McCrea, J. M. (1950). On the Isotopic Chemistry of Carbonates and a Paleotemperature Scale. *The Journal of Chemical Physics*, 18(6), 849–857. doi: <http://dx.doi.org/10.1063/1.1747785>
- Millero, F. J. (1974). Seawater as a multicomponent electrolyte solution. *The sea*, 5, 3–80.
- Morse, J. W., & Bender, M. L. (1990). Partition coefficients in calcite: Examination of factors influencing the validity of experimental results and their application to natural systems. *Chemical Geology*, 82, 265–277. doi: [http://dx.doi.org/10.1016/0009-2541\(90\)90085-L](http://dx.doi.org/10.1016/0009-2541(90)90085-L)
- Nielsen, L. C., DePaolo, D. J., & De Yoreo, J. J. (2012). Self-consistent ion-by-ion growth model for kinetic isotopic fractionation during calcite precipitation. *Geochimica et Cosmochimica Acta*, 86, 166–181. doi: <http://dx.doi.org/10.1016/j.gca.2012.02.009>
- Pak, D. K., Lea, D. W., & Kennett, J. P. (2004). Seasonal and interannual variation in Santa Barbara Basin water temperatures observed in sediment trap foraminiferal Mg/Ca. *Geochemistry, Geophysics, Geosystems*, 5(12), n/a–n/a. doi: 10.1029/2004GC000760
- Reeder, R. J., Nugent, M., Lamble, G. M., Tait, C. D., & Morris, D. E. (2000). Uranyl Incorporation into Calcite and Aragonite: XAFS and Luminescence Studies. *Environmental Science & Technology*, 34(4), 638–644. doi: 10.1021/es990981j
- Rink, S., Kühl, M., Bijma, J., & Spero, H. J. (1998). Microsensor studies of photosynthesis and respiration in the symbiotic foraminifer *Orbulina universa*. *Marine Biology*, 131(4), 583–595. doi: 10.1007/s002270050350
- Russell, A. D., Hönisch, B., Spero, H. J., & Lea, D. W. (2004). Effects of seawater carbonate ion concentration and temperature on shell U, Mg, and Sr in cultured planktonic foraminifera. *Geochimica et Cosmochimica Acta*, 68(21), 4347–4361. doi: <http://dx.doi.org/10.1016/j.gca.2004.03.013>

- Sadekov, A. Y., Eggins, S. M., & De Deckker, P. (2005). Characterization of Mg/Ca distributions in planktonic foraminifera species by electron microprobe mapping. *Geochemistry, Geophysics, Geosystems*, 6(12), n/a-n/a. doi: 10.1029/2005GC000973
- Sanyal, A., Hemming, N. G., Broecker, W. S., Lea, D. W., Spero, H. J., & Hanson, G. N. (1996). Oceanic pH control on the boron isotopic composition of foraminifera: Evidence from culture experiments. *Paleoceanography*, 11(5), 513-517. doi: 10.1029/96PA01858
- Sanyal, A., Nugent, M., Reeder, R. J., & Bijma, J. (2000). Seawater pH control on the boron isotopic composition of calcite: evidence from inorganic calcite precipitation experiments. *Geochimica et Cosmochimica Acta*, 64(9), 1551-1555. doi: [http://dx.doi.org/10.1016/S0016-7037\(99\)00437-8](http://dx.doi.org/10.1016/S0016-7037(99)00437-8)
- Spero, H. J. (1988). Ultrastructural examination of chamber morphogenesis and biomineralization in the planktonic foraminifer *Orbulina universa*. *Marine Biology*, 99(1), 9-20. doi: 10.1007/BF00644972
- Spero, H. J., Eggins, S. M., Russell, A. D., Vetter, L., Kilburn, M. R., & Hönisch, B. (2015). Timing and mechanism for intratest Mg/Ca variability in a living planktic foraminifer. *Earth and Planetary Science Letters*, 409, 32-42. doi: <http://dx.doi.org/10.1016/j.epsl.2014.10.030>
- Spero, H. J., & Parker, S. L. (1985). Photosynthesis in the symbiotic planktonic foraminifer *Orbulina universa*, and its potential contribution to oceanic primary productivity. *The Journal of Foraminiferal Research*, 15(4), 273-281. doi: 10.2113/gsjfr.15.4.273
- Swart, P. K., & Hubbard, J. A. E. B. (1982). Uranium in scleractinian coral skeletons. *Coral Reefs*, 1(1), 13-19. doi: 10.1007/BF00286535
- Takagi, H., Kimoto, K., Fujiki, T., Kurasawa, A., Moriya, K., & Hirano, H. (2016). Ontogenetic dynamics of photosymbiosis in cultured planktic foraminifers revealed by fast repetition rate fluorometry. *Marine Micropaleontology*, 122, 44-52. doi: <http://dx.doi.org/10.1016/j.marmicro.2015.10.003>
- Tang, J., Köhler, S. J., & Dietzel, M. (2008). Sr²⁺/Ca²⁺ and ⁴⁴Ca/⁴⁰Ca fractionation during inorganic calcite formation: I. Sr incorporation. *Geochimica et Cosmochimica Acta*, 72(15), 3718-3732. doi: <http://dx.doi.org/10.1016/j.gca.2008.05.031>
- Tesoriero, A. J., & Pankow, J. F. (1996). Solid solution partitioning of Sr²⁺, Ba²⁺, and Cd²⁺ to calcite. *Geochimica et Cosmochimica Acta*, 60(6), 1053-1063. doi: [http://dx.doi.org/10.1016/0016-7037\(95\)00449-1](http://dx.doi.org/10.1016/0016-7037(95)00449-1)
- Uchikawa, J., Penman, D. E., Zachos, J. C., & Zeebe, R. E. (2015). Experimental evidence for kinetic effects on B/Ca in synthetic calcite: Implications for potential B(OH)₄⁻ and B(OH)₃ incorporation. *Geochimica et Cosmochimica Acta*, 150, 171-191. doi: <http://dx.doi.org/10.1016/j.gca.2014.11.022>
- Urey, H. C., Lowenstam, H. A., Epstein, S., & McKinney, C. R. (1951). Measurement of paleotemperatures and temperatures of the upper Cretaceous of England, Denmark and the Southeastern United States. *Geological Society of America Bulletin*, 62(4), 399-416. doi: 10.1130/0016-7606(1951)62[399:mopato]2.0.co;2
- Van Heuven, S., Pierrot, D., Lewis, E., & Wallace, D. (2011). MATLAB Program Developed for CO₂ System Calculations. ORNL/CDIAC-105b. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, Tennessee.
- Walker, R. A. (2004). Photosynthesis and respiration in five species of benthic foraminifera that host algal symbionts. *Graduate Theses and Dissertations, University of South Florida*. doi: <http://scholarcommons.usf.edu/etd/1287>

- Weiner, S., & Dove, P. M. (2003). An Overview of Biomineralization Processes and the Problem of the Vital Effect. *Reviews in Mineralogy and Geochemistry*, 54(1), 1-29. doi: 10.2113/0540001
- Wolf-Gladrow, D., & Riebesell, U. (1997). Diffusion and reactions in the vicinity of plankton: A refined model for inorganic carbon transport. *Marine Chemistry*, 59(1-2), 17-34. doi: [http://dx.doi.org/10.1016/S0304-4203\(97\)00069-8](http://dx.doi.org/10.1016/S0304-4203(97)00069-8)
- Wolf-Gladrow, D. A., Bijma, J., & Zeebe, R. E. (1999). Model simulation of the carbonate chemistry in the microenvironment of symbiont bearing foraminifera. *Marine Chemistry*, 64(3), 181-198. doi: [http://dx.doi.org/10.1016/S0304-4203\(98\)00074-7](http://dx.doi.org/10.1016/S0304-4203(98)00074-7)
- Wolthers, M., Nehrke, G., Gustafsson, J. P., & Van Cappellen, P. (2012). Calcite growth kinetics: Modeling the effect of solution stoichiometry. *Geochimica et Cosmochimica Acta*, 77, 121-134. doi: <http://dx.doi.org/10.1016/j.gca.2011.11.003>
- Yu, J., Elderfield, H., & Hönisch, B. (2007). B/Ca in planktonic foraminifera as a proxy for surface seawater pH. *Paleoceanography*, 22(2), n/a-n/a. doi: 10.1029/2006PA001347
- Zeebe, R. E., Bijma, J., & Wolf-Gladrow, D. A. (1999). A diffusion-reaction model of carbon isotope fractionation in foraminifera. *Marine Chemistry*, 64(3), 199-227. doi: [http://dx.doi.org/10.1016/S0304-4203\(98\)00075-9](http://dx.doi.org/10.1016/S0304-4203(98)00075-9)
- Zeebe, R. E., & Sanyal, A. (2002). Comparison of two potential strategies of planktonic foraminifera for house building: Mg²⁺ or H⁺ removal? *Geochimica et Cosmochimica Acta*, 66(7), 1159-1169. doi: [http://dx.doi.org/10.1016/S0016-7037\(01\)00852-3](http://dx.doi.org/10.1016/S0016-7037(01)00852-3)
- Zeebe, R. E., Wolf-Gladrow, D. A., Bijma, J., & Hönisch, B. (2003). Vital effects in foraminifera do not compromise the use of $\delta^{11}\text{B}$ as a paleo-pH indicator: Evidence from modeling. *Paleoceanography*, 18(2). doi: 10.1029/2003PA000881

Figure captions

Fig. 1. A schematic cross section through *O. universa* that distinguishes the ambient seawater from the chemical microenvironment and the internal calcification environment. The chemical microenvironment is modified from the external seawater on a diurnal timescale by symbiont photosynthetic activity, respiration and calcification. Seawater might be endocytosed and transported from the chemical microenvironment to the calcification environment, or there might be more open communication between the calcification environment and the chemical microenvironment. Adapted from Erez, (2003) and de Nooijer et al. (2014).

Fig. 2. a) Seawater carbonate chemistry changes predicted to occur immediately adjacent to the shell wall of *O. universa* over multiple diurnal cycles. The model results shown are calculated using the diffusion-reaction model of Zeebe et al. (2003) and fit to a sinusoidal curve that matches the full range of chemistry calculated for light and dark conditions assuming ambient seawater conditions, $\text{pH}_{\text{TOTAL}} \approx 8.00$ $\text{DIC} \approx 2000 \mu\text{mol kg}^{-1}$, and a foraminifer shell radius of $250\mu\text{m}$. Rates of photosynthesis, respiration and calcification were fixed at 10, 2 and 3 nmol C h^{-1} in the light, and 0, 2 and 1 nmol C h^{-1} in the dark, following Zeebe et al. (2003). b) The diurnal changes in microenvironment carbonate chemistry are converted to changes in the B/Ca (solid line) and U/Ca (dashed line) composition of shell calcite precipitated by *O. universa* using published bulk shell proxy calibrations for B/Ca (Allen et al., 2011) and U/Ca ratios (Russell et al., 2004).

Fig. 3. LA-ICP-MS transects through individual *O. universa* shells grown in culture experiments. Mg/Ca (black), B/Ca (blue) and U/Ca (red) profiles were measured from the

inside through to the outside surface of the shell wall. Mg/Ca banding provides a reference for day-night calcification (Spero et al., 2015), as demonstrated in dark/light annotations (e.g. panel (a)), the bands are numbered for each day after sphere formation. In general, B/Ca bands are broadly out of phase with Mg/Ca bands. Where present, U/Ca bands are broadly in phase with Mg/Ca bands (profiles a-d). U/Ca is also notable for often being elevated at the POS. Profiles are grouped by the experiment seawater type and labelled with the foraminifer identification number. The small dots represent the measurement noise associated with each TE/Ca profile. Note that the sample in panel (g) was placed into high pH experimental seawater only after it had produced a thinly calcified adult sphere. As a result, its shell composition near the POS has low B/Ca ($<500 \mu\text{mol/mol}$) and low U/Ca, which reflects the lower B and U concentrations in natural seawater compared to the experimental seawater.

Fig. 4. LA-ICP-MS depth profiles were de-trended using a Butterworth bandpass Fourier filter, in order to isolate banding patterns from low frequency trends and from high frequency noise. Plotted are the de-trended depth profiles of Mg/Ca (black), B/Ca (blue) and U/Ca (red) through shells of *O. universa*. Profiles are displayed from the inside through to the outside surface of the shell wall. The sample number of each foraminifer is noted in the top right corner.

Fig. 5. LA-ICP-MS profiles of Mg/Ca (black), Sr/Ca (orange) and Mn/Ca (purple) through chamber walls of individual *O. universa* cultured in this study (refer to the caption of Fig. 3 for the details of plot labelling). Note the timing of Mn/Ca variations with Mg/Ca banding in low DIC experiments, (a-b). Sr/Ca ratios tend to be higher on the outside of the POS relative to the inside.

759

760 **Fig. 6.** Bulk shell Mg/Ca, Sr/Ca, B/Ca, Mn/Ca and U/Ca ratio values of *O. universa* vs.
761 seawater carbonate system parameters (circles). Error bars (1σ) reflect variability of
762 individual shell compositions (crosses) grown within each experiment. Arrows indicate
763 correction of shell B/Ca, Mn/Ca and U/Ca compositions to account for the 2.6x lower
764 $[B]_T$, 4.7x higher $[Mn]_T$, and 3.1x lower $[U]_T$ of the low DIC experiment (see text for details).
765 The adjustment of $[B]_T$ and $[U]_T$ of the low DIC experiment up to the elevated $[B]_T$ and
766 $[U]_T$ of the other experiments is made to emphasize the elevated boron and uranium
767 concentrations we have used in these experiments. Thick black lines show significant linear
768 regression fits obtained between bulk shell trace element to calcium ratios and the
769 various seawater carbonate system parameters. Grey lines show scaled relationships for bulk
770 shell compositions reported by Allen et al. (2011) for B/Ca, and by Russell et al. (2004) for
771 U/Ca, and the relationship between Mg/Ca with $[CO_3^{2-}]$ and pH from Russell et al. (2004).

772

773 **Fig. 7.** Variation of apparent partition coefficients, $K_{D\_B/Ca}$, $K_{D\_Mg/Ca}$, $K_{D\_Mn/Ca}$, $K_{D\_Sr/Ca}$, and
774 $K_{D\_U/Ca}$ with linear calcification rate (reported as $\log_{10}(\text{Rate})$ in $\text{mol m}^{-2} \text{s}^{-1}$). Error bars (1σ)
775 reflect the variability of individual shell compositions and calcification rates in each
776 experiment.

777

778 **Fig. 8.** De-trended (see text) and predicted B/Ca (blue) and U/Ca (red) from the inner shell
779 surface through to the outside of the shell wall in individual *O. universa* from culture
780 experiments. Profiles are grouped by the experiment seawater type and labelled with the
781 foraminifer identification number. In these plots the axis scales for B/Ca and U/Ca are
782 consistent throughout, to enable comparison of shell responses to different seawater
783 chemistries and modelling predictions. Panels (i) and (j) show examples of changes in

predicted B/Ca and U/Ca in sample #2001, if calcification rate and photosynthesis inputs to the diffusion-reaction model were doubled and halved. Also plotted in panels (i) and (j) is the de-trended profile of sample #2004.

Fig. 9. a) Bulk shell B/Ca of *O. universa* plotted as a function of seawater $[\text{B}(\text{OH})_4^-]/[\text{HCO}_3^-]$ ratio. The low DIC experiments had $[\text{B}]_{\text{T}}$ less than half that of the other experiments, requiring its measured bulk shell B/Ca (open symbol) to be corrected using the B/Ca - $[\text{B}(\text{OH})_4^-]$ relationship from Allen et al. (2011). b) A comparison of the B/Ca results from our experiments and Allen et al. (2011), including a correction to the Allen et al. (2011) pH experiments for 5x lower $[\text{B}]_{\text{T}}$. Error bars are $\pm 2\text{se}$. The trends between the data in this study and those of Allen et al. (2011) are broadly comparable, the variability in our data is a result of the small number of foraminifers used to produce mean shell B/Ca for each experiment.

Table 1. Experimental conditions for foraminiferal culturing experiments, at $21.92 \pm 0.13^\circ\text{C}$, and 33.3 ± 0.2 psu. pH_{TOTAL} , DIC, $[\text{HCO}_3^-]$, $[\text{CO}_3^{2-}]$, $[\text{B}(\text{OH})_4^-]$ and Ω_{Ca} are calculated at 22°C from total alkalinity, pH_{NBS} and $[\text{B}]_{\text{T}}$ measurements, made at 25°C using a modified version of the van Heuven et al. (2011) Matlab script, CO2SYS.m. Uncertainties associated with the calculated carbonate system parameters are attained with Monte Carlo simulations of CO2SYS using the measurement errors of the input total alkalinity ($\leq 36 \mu\text{mol kg}^{-1}$), pH_{NBS} (≤ 0.02) and $[\text{B}]_{\text{T}}$ (1.25 %).

Experiment Type	No. Forams	pH _{TOTAL}		DIC		Alkalinity		HCO ₃ ⁻		CO ₃ ²⁻		B(OH) ₄ ⁻		Ω	
		2σ		μmol kg ⁻¹	2σ	μmol kg ⁻¹	2σ	μmol kg ⁻¹	2σ	μmol kg ⁻¹	2σ	μmol kg ⁻¹	2σ	2σ	
Low pH	2	7.53	0.01	2047	34	2214	36	1943	33	61	2	148	5	1.5	0.1
Low DIC	3	7.96	0.01	1026	5	1230	2	940	6	78	1	129	4	1.9	0.1
High DIC	3	7.97	0.02	4019	29	4671	10	3673	37	315	11	362	16	7.8	0.3
High pH	3	8.44	0.02	2135	39	3380	5	1696	44	434	10	802	33	10.7	0.3

Figure
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