1 **Trace element proxies for surface ocean conditions:**
2 **A synthesis of culture calibrations with planktic foraminifera**
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14 **Abstract**
15

16 The trace element composition of planktic foraminiferal calcite provides a useful means
17 of determining past surface ocean conditions. We have assembled the results of culture
18 experiments for three species of symbiont-bearing planktic foraminifera, *Globigerinoides*
19 *ruber*, *Globigerinoides sacculifer*, and *Orbulina universa*, and one symbiont-barren
20 species, *Globigerina bulloides*, to evaluate their responses to temperature, salinity, pH,
21 carbonate ion, and dissolved inorganic carbon (DIC) growth conditions. Trace element
22 ratios (Li/Ca, B/Ca, Mg/Ca, Sr/Ca, Mn/Ca, Cd/Ca, Ba/Ca, Na/Ca, and U/Ca) were
23 measured simultaneously on samples grown with the same culture techniques, which
24 provides robust, reliable calibrations that may be used together in multi-proxy
25 paleoceanographic studies. Our data confirm that temperature is the dominant control on
26 foraminiferal Mg/Ca under the ranges of conditions studied and that the potential effects
27 of salinity and CO₃²⁻ on Mg/Ca of these tropical species across late Pleistocene glacial
28 cycles are relatively small. Carbonate system experiments suggest that Sr/Ca may be
29 useful for reconstructing large DIC changes. Na/Ca increases with salinity in *G. ruber*
30 (pink), but not in *G. sacculifer*. As these emerging proxy relationships become more
31 firmly established, the synthesis of multiple trace element ratios may help
32 paleoceanographers isolate the effects of different environmental parameters in paleo
33 records. Calcification rates (μg/day) vary among species and do not respond consistently
34 to any experimental parameter. Comparison of our calcification rates with those observed
35 in inorganic calcite precipitation experiments suggest that foraminifera calcify ~100x
36 more slowly than inorganic calcites grown in similar solutions. We suggest that
37 calcification rate does not typically exert a dominant control on trace element partitioning
38 in planktic foraminiferal calcite, though it may play a role for some elements under
39 certain circumstances. Differences in average growth rate cannot explain composition
40 differences among species, pointing to alternative controls that may be biological in
41 origin.
42

43 **1 Introduction**
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45 Shells (tests) of foraminifera preserved on the sea floor constitute an important archive
46 for paleoceanographers, and much has been done in recent decades to quantify and
47 understand relationships between the composition of foraminiferal calcite and the

environmental conditions in which foraminifers have grown. The shell of modern planktic foraminifera is composed of calcite, the trigonal polymorph of CaCO_3 . Calcite's flexible corner-sharing structure allows it to accommodate a wide range of trace elements (Reeder, 1983), but the total contribution of trace elements to foraminiferal calcite is typically less than 2 % (moles of trace elements / moles calcium x 100), including Li, B, F, Na, Mg, P, V, Mn, Fe, Cu, Zn, Sr, Cd, Ba, Nd, and U (Lea, 1999). Laboratory and field studies have shown that changes in environmental conditions such as temperature and seawater carbonate ion concentration ($[\text{CO}_3^{2-}]$) can produce predictable variations in the trace element composition of foraminiferal calcite (e.g., Anand et al., 2003; Lea et al., 1999; Russell et al., 2004). Assuming that fossil foraminifera behaved similarly to these modern specimens, the calibration of these variations can be used to infer past environmental changes.

In this study we report the chemical composition of calcite precipitated in culture experiments by planktic foraminifera, specifically the ratios of lithium, magnesium, strontium, manganese, cadmium, sodium, and uranium to calcium (Li/Ca, Mg/Ca, Sr/Ca, Mn/Ca, Cd/Ca, Na/Ca, and U/Ca). In previous studies, these ratios have often been considered separately, and one of our goals is to consider a wide variety of trace elements to gain new insight into these proxies. Some models of trace element incorporation predict different partitioning behavior for different elements due to variations in chemical properties (e.g., ionic radius, charge). Therefore a holistic approach that includes a wide range of elements enables testing of these predictions. For example, Elderfield et al. (1996) analyzed a suite of trace element ratios to test the idea that Rayleigh distillation from an internal pool or reservoir could explain trace element partitioning during foraminiferal calcification. Their results provided some support for this model in benthic foraminifera, but not in planktics. Later studies built on the pioneering work of Elderfield et al. by considering the distribution of multiple elements within single shells (e.g., Hathorne et al., 2009), which confirmed the earlier studies' findings regarding the lack of Rayleigh-distillation behavior in planktics and also provided a detailed picture of micro-scale trace element distribution patterns.

Here, we synthesize a suite of trace element and calcification rate data to assess controls on the composition of foraminiferal calcite. We aim to assess whether some recent models for trace element incorporation in inorganic carbonates may apply to foraminifera as well. According to one growth-entrapment model of trace element incorporation (DePaolo, 2011), as mineral growth rates increase, incorporation of cations larger than Ca^{2+} (hereafter "large" ions) should increase while incorporation of cations smaller than Ca^{2+} ("small" ions) should decrease. The underlying idea is that larger ions are easier to dehydrate, which might lead to greater rates of incorporation compared to smaller, more strongly hydrated ions at higher growth rates. Another growth-entrapment model is based on the idea that trace element incorporation is determined by the competing effects of crystal growth rate and diffusivity of ions in the near-surface region of a growing crystal (Watson, 1996 and 2004). These two models invoke different mechanisms but produce similar predictions regarding trace metal inclusion in calcite. If such growth-rate effects exerted a dominant influence on foraminifera, we should see an inverse relationship between concentrations of large cations (Sr^{2+} , Ba^{2+}) and small cations (Mg^{2+} , Mn^{2+} , Cd^{2+})

in foraminiferal calcite. Our large suite of trace element data measured on the same samples allows this kind of hypothesis to be tested.

First, to provide context for our new results, we summarize existing knowledge and observations from both field-based studies and laboratory-based culture experiments.

1.1 Large divalent cations

Sr/Ca

Both culture and field studies demonstrate Sr/Ca of planktonic foraminiferal calcite is not influenced strongly by temperature or salinity (e.g., Lea et al. 1999), but do suggest increases with shell calcification rate (Dueñas-Bohórquez et al., 2009; Elderfield et al., 2002; Kısakürek et al., 2008; Lea et al., 1999b). This is consistent with observations of increased Sr incorporation in calcite at higher precipitation rates in inorganic precipitation studies (Gabitov and Watson, 2006; Lorens, 1981; Paquette and Reeder, 1995), and suggests observed increases with temperature and salinity in planktic foraminifer shells may be an indirect effect of enhanced calcification rate (Dueñas-Bohórquez et al., 2009). Systematic variations in planktic foraminifer shell Sr/Ca in the geologic record have been attributed to changes in seawater Sr/Ca, and also to $[\text{CO}_3^{2-}]$ and nutrient availability (Billups et al., 2004; Martin et al., 1999; Stoll et al., 1999). As with temperature and salinity, these latter factors might be linked to their influence on foraminifer calcification rates. Sr/Ca varies little within single shells (Hathorne et al., 2009).

Ba/Ca

Previous culture studies have demonstrated that Ba/Ca of foraminiferal calcite exhibits no detectable response to salinity, temperature, or carbonate system parameters; rather, it increases linearly with Ba/Ca of seawater (Lea and Spero, 1994; Hönisch et al., 2011). Empirical evidence for the influence of growth rate on Ba/Ca of inorganic calcite is mixed, with some experiments suggesting an increase in Ba incorporation with growth rate (Tesoriero and Pankow, 1996) and others suggesting Ba incorporation is independent of growth rate (Pingitore and Eastman, 1984). As a result of its strong dependence on Ba/Ca of seawater, Ba/Ca of foraminiferal calcite has been used to track ocean processes that involve changes in seawater Ba/Ca, such as freshwater runoff (which has much higher [Ba] than seawater) and remineralization of Ba-rich biogenic particles in the deep ocean (e.g., Hall and Chan, 2004). Since results of our culture experiments have been thoroughly discussed in Hönisch et al. (2011), we only discuss them here in the context of the trace element suite.

1.2 Small divalent cations

Cd/Ca

Based on a core-top study in the North Atlantic, Rickaby and Elderfield (1999) suggested that Cd incorporation in some species is influenced by temperature. The effect of salinity has not yet been extensively tested. Experiments with inorganic calcite suggest that Cd incorporation decreases as growth rate increases (Lorens, 1981; Tesoriero and Pankow, 1996). Cd/Ca in planktic foraminifera has been used as a proxy for phosphate concentration in seawater (Boyle, 1988; Delaney, 1989; Yu et al., 2013). This is based on the strong correlation that exists between Cd and phosphate and also $\delta^{13}\text{C}$ in the modern ocean. The response of planktonic foraminiferal Cd/Ca to seawater nutrients is species-specific. *Globigerina bulloides*' Cd/Ca appears to reflect seawater Cd concentrations (Mashiotta et al., 1997), while symbiont-bearing species are characterized by much lower Cd incorporation (Mashiotta et al., 1997; Ripperger et al., 2008).

Mn/Ca

Temperature, salinity, and carbonate system influences on Mn/Ca incorporation in planktonic foraminifera have not previously been tested in culture experiments. In inorganic experiments, Mn/Ca of calcite overgrowths has been observed to increase with precipitation rate (Mucci, 1988). The amount of Mn dissolved in seawater is influenced by terrestrial input and redox reactions, and Mn/Ca of foraminiferal calcite has been used to infer changes in these processes across the last glacial cycle (Klinkhammer et al., 2009). Based on core-top samples, Mn/Ca has also been interpreted to reflect nutrient content of surface waters (Marr et al., 2013).

Mg/Ca

Field-based and laboratory culture studies have consistently shown Mg/Ca to increase with temperature at a rate of 8-10% per °C (Delaney et al., 1985; Lea et al., 1999b; Kısakürek et al., 2008) and to increase with salinity (Ferguson et al., 2008; Dueñas-Bohórquez et al., 2009; Hönisch et al., 2013; Kısakürek et al., 2008; Lea et al., 1999b; Nürnberg et al., 1996). These studies provide the foundation for using foraminiferal Mg/Ca as a highly sensitive temperature proxy. Culture experiments have further revealed the Mg/Ca composition of *O. universa* and *G. bulloides* to decrease as seawater pH and $[\text{CO}_3^{2-}]$ increase (Evans et al., 2016; Kısakürek et al., 2008; Lea et al., 1999b; Russell et al., 2004). Russell et al. (2004) suggested that the effect becomes negligible above a certain $[\text{CO}_3^{2-}]$ threshold. In a recent compilation of Mg/Ca data from culture experiments, Evans et al. (2016) find that Mg/Ca decreases linearly with $[\text{CO}_3^{2-}]$ with no threshold and they also provide a logistic fit for the Mg/Ca-pH relationship that includes two inflection points. Because pH and $[\text{CO}_3^{2-}]$ covary in all of these experiments, it has not yet been possible to distinguish between a pH and a $[\text{CO}_3^{2-}]$ control. Relative Mg/Ca enrichment at low pH could be the result of reduced efficiency of Mg removal from the calcifying fluid in the presence of higher $[\text{H}^+]$ (Evans et al., 2015). Results of culture experiments are notably different from inorganic calcite precipitation experiments, which produce much smaller Mg/Ca sensitivity to temperature (ca. 2-3% per °C) and much higher Mg/Ca calcite ratios when precipitation occurs from seawater (by 1 to 2 orders of magnitude) (reviewed in Lea, 2003).

Mg/Ca in planktic foraminiferal calcite is typically 1-2 orders of magnitude lower than that of inorganic calcite (e.g., Mucci and Morse, 1983; Oomori et al., 1987; Hartley and Mucci, 1996), and it has been suggested that foraminifera may actively remove Mg ions from calcification fluids via trans-membrane channels or pumps (Zeebe and Sanyal, 2002; Bentov and Erez, 2006). Intrashell variation in a series of high/low (night/day) Mg bands may be related to variable Mg uptake by mitochondria (Spero et al., 2015).

1.3 Other ions

U/Ca

No significant U/Ca-temperature relationship has been observed in culture experiments, but Yu et al. (2008) report a positive correlation between U/Ca and temperature for *Globigerina bulloides*, *Neogloboquadrina pachyderma*, and *Globorotalia inflata* in core-top samples from the North Atlantic. Evidence from previous culture experiments suggests U/Ca decreases with $[\text{CO}_3^{2-}]$ and pH (Russell et al., 2004), and U/Ca has been observed to decrease with $[\text{CO}_3^{2-}]$ in benthic foraminifera as well (Raitzsch et al., 2011; Keul et al., 2013). These relationships are broadly consistent with the incorporation of uranyl ion (UO_2^{2+}) as rutherfordine (UO_2CO_3) into calcite, which is the result of changes in uranium speciation with $[\text{CO}_3^{2-}]$ and pH in seawater (e.g., Djogić and Branica, 1991). Results of inorganic calcite growth experiments suggest that U incorporation is strongly influenced by properties of the crystal surface, with a possible role for growth rate (Reeder et al., 2001).

Li/Ca

Temperature does not exert a measurable effect on Li/Ca in cultured *G. sacculifer* (Delaney and Bé, 1985). Field investigations of a temperature-Li/Ca link yield different results for different species. Temperature was ruled out as a key control on Li/Ca in *O. universa* based on a study of North Atlantic core-top and down-core samples, from which pressure and dissolution could also be eliminated as factors (Hall and Chan, 2004), while in *N. pachyderma* (s), Li/Ca increases linearly with temperature (Hendry et al., 2009). It is not clear whether salinity exerts control on Li/Ca of planktic foraminifera, but in inorganic calcite, Li/Ca has been observed to increase four-fold between salinities of 10 and 50 (Marriott et al., 2004), which encompasses a much larger range than typical seawater variations. Early culture experiments indicated Li/Ca in planktic foraminifer shells responded to seawater Li/Ca, though not in a strictly linear fashion (Delaney and Bé, 1985). This relationship was subsequently applied to the fossil record to infer changes in seafloor hydrothermal fluxes and oceanic crust formation rates (Delaney and Boyle, 1986). Later work revealed systematic foraminiferal Li/Ca variations across late Pleistocene glacial cycles, which prompted a search for additional controls on Li incorporation because the observed changes were too rapid to be explained by plausible rates of change in the ocean's Li inventory (Hall and Chan, 2004; Hall et al., 2005). Instead, Hall and Chan (2004) proposed a $[\text{CO}_3^{2-}]$ -driven growth rate control on Li incorporation, but this relationship has not yet been tested in laboratory culture.

Na/Ca

Na/Ca has been proposed as a proxy for salinity based on culture experiments with the benthic foraminifer *Ammonia tepida* (Wit et al., 2013), but no systematic relationships have yet been reported for planktic foraminifera, and the influence of temperature has not been tested. The Na content of some natural carbonates including limestone, travertine, and invertebrate shells reflects seawater salinity (Gordon et al., 1970; Ichikuni and Kikuchi, 1972; Sakae et al., 1979), but the positive correlation is only robust between 0 and 10 salinity units (Ishikawa and Ichikuni, 1984). The free Na ion is univalent (+1) and its incorporation is thought to proceed differently from many of the elements discussed above, which substitute directly for Ca^{2+} as divalent (+2) cations in the calcite lattice (this may also apply to Li^+). In inorganic calcites, Na occupies interstitial positions between carbonate ions and CaO_6 octahedra (Busenberg and Plummer, 1985; Ishikawa and Ichikuni, 1984; White, 1978), and the amount of Na incorporated varies as a function of crystal growth rate, possibly because the number of crystal defects (and interstitial spaces) increases as growth rates increase (Busenberg and Plummer, 1985).

B/Ca

In shells of cultured planktonic foraminifera, B/Ca increases with pH of the culture solution (e.g., Sanyal et al., 2001; Allen et al., 2011; Allen et al., 2012; Hennehan et al., 2015), broadly consistent with the prediction that B incorporation should increase as seawater borate (B(OH)_4^-) concentrations increase (Hemming and Hanson, 1992). B/Ca is not only sensitive to B(OH)_4^- , but to the relative abundance of B(OH)_4^- and dissolved inorganic carbonate species, $\text{B(OH)}_4^-/\text{HCO}_3^-$ or perhaps $\text{B(OH)}_4^-/\text{DIC}$ (Yu et al., 2007; Allen et al., 2012). In cultured *O. universa*, *G. ruber* and *G. sacculifer*, B/Ca increases linearly with salinity and total seawater B concentration (B_T), but exhibits no significant response to temperature (Allen et al., 2012). B/Ca of core-top *G. inflata* and *G. bulloides* increases with temperature (Yu et al., 2007); however, covariation of temperature with salinity and carbonate system parameters in these surface waters precludes a clear assessment of a B/Ca-temperature relationship based on core-top data alone (Allen and Hönisch, 2012). Results from inorganic calcite growth experiments suggest that B/Ca increases with growth rate and with $\text{B}_\text{T}/\text{DIC}$ (Uchikawa et al., 2015). Samples from Bermuda sediment traps reveal that B/Ca in *G. ruber* (white) varies seasonally, perhaps as a result of shifting micro-environment chemistry driven by variable symbiont activity (Babila et al., 2014). Hennehan et al. (2015) find a positive correlation between B/Ca and seawater phosphate (PO_4^{3-}) based on *G. ruber* (white) core-top data; the influence of nutrient concentrations merits further investigation.

By systematically altering temperature, salinity, pH and DIC growth conditions in a suite of laboratory culture experiments, we aim to improve our understanding of controls on incorporation of these trace elements in planktic foraminiferal calcite. In the following discussion, we synthesize a multi-element dataset and compare our results with other studies to test and extend existing proxy calibrations.

2 Methods

2.1 Culture experiments

This study incorporates new results from experiments conducted on Santa Catalina Island and Puerto Rico (*G. ruber*, *G. sacculifer*, *O. universa*, and *G. bulloides*) and previously reported results from Allen et al. (2011) (*O. universa*) and Allen et al. (2012) (*G. ruber* (pink) and *G. sacculifer*). Culture experiments and analytical methods are summarized below; detailed descriptions are provided in the previous publications.

Culture experiments were performed at the Wrigley Marine Science Center on Santa Catalina Island (33°26'40"N, 118°28'55"W) during summers 2000, 2008, and 2013, and at the University of Puerto Rico's Marine Sciences Center on Isla Magueyes (collection site 17°52'N, 66°58'W) during March and April 2010. Juvenile foraminifers were hand-collected by SCUBA divers in open ocean conditions either two (Catalina Island) or eight (Puerto Rico) nautical miles offshore and between 2 and 8 meters depth. Immediately after collection, samples were brought to the laboratory, where the foraminifera were identified and their maximum shell diameters were determined using inverted light microscopes. Specimens were then transferred to individual 120 mL soda lime glass jars filled with experimental seawater and sealed with snap lids to prevent gas exchange with the atmosphere. Seawater used in these culture experiments was collected offshore and filtered (0.8 μ m) to remove large particles, with the exception of the low-DIC experiment that consisted of half natural (filtered) and half artificial seawater (MilliQ water plus salts (see Allen et al., 2012). Experiments were designed to vary either temperature, salinity, or carbonate system parameters separately. Approximately 20 *O. universa*, 20 *G. sacculifer*, and 50 *G. ruber* (pink) specimens were cultured for each experimental condition, and individually fed a one-day-old brine shrimp nauplius (*Artemia salina*) every other day. Cultures were maintained in temperature-controlled water baths and illuminated using cool white fluorescent lamps on a strict diurnal cycle: 12 hours light, 12 hours dark. Over the course of 1 – 3 weeks, foraminifers grew new chambers on their calcite shells to accommodate cytoplasm growth. Experiments ended after a specimen underwent gametogenesis. The remaining empty calcite shell was then rinsed in de-ionized water, dried, and archived for later analysis. The maximum shell diameter after gametogenesis was measured to determine total growth under the experimental conditions.

The desired pH (7.5 – 8.6, total scale) of experimental seawater was set by titrating filtered seawater with NaOH or ultrapure HCl. Salinity was varied between 30 and 40 $\pm$ 0.1 by diluting seawater with deionized water or by partial evaporation under a heat lamp at 60°C. Culture jars were placed in separate, temperature-controlled water baths, the temperatures of which were set to specific values that ranged between 17.7 $\pm$ 0.5 and 29.3 $\pm$ 0.2 °C ($\pm$ 1 σ). To monitor for CO₂ exchange with the laboratory atmosphere during feeding (when caps must be removed), as well as the net effects of respiration, photosynthesis and calcification, alkalinity and pH were measured at the beginning and end of each experiment using a Metrohm 809 open cell auto-titrator and pH meter, calibrated with NIST buffers and Dickson-certified alkalinity standards. After analysis,

pH values measured on the NBS scale were converted to the total scale according to the standard equation (Zeebe and Wolf-Gladrow, 2001).

Additional culture experiments with *O. universa* were carried out during July and August of 2013 on Santa Catalina Island to investigate proxy relationships under Paleocene seawater chemistry. Sample collection and experimental methods follow procedures nearly identical to those above. Experimental seawater batches were composed of half artificial and half natural (filtered) seawater, but concentrations of Ca, Mg, and B were modified to reflect estimated Paleocene values (20 mmol/kg, 30 mmol/kg, and 365 μ mol/kg, respectively, at a salinity of 33.1-33.3 see Table S1) (Tyrell and Zeebe 2004, Lemarchand et al. 2000). Resultant Sr/Ca_{SW} values were thus roughly half of modern (4.58 mmol/mol on average). DIC was varied independently from pH by the addition of NaHCO₃ to reach target values ranging from 1000-4000 μ mol/kg.

2.2 Carbonate system calculation

The Matlab program CO2SYS.m was used to calculate carbonate system parameters from measured values of seawater pH, alkalinity, temperature and salinity (van Heuven, 2011). Values presented in Table S1 are averages determined for each experiment (n $\approx$ 10 measurements per batch of seawater). Carbonate system calculations assume atmospheric pressure (1 atm), Mehrbach et al. (1973) constants for the dissociation of carbonic acid and bicarbonate, K1 and K2, refit by Lueker et al. (2000) on the total pH scale, KSO₄ dissociation constant recommended by Dickson et al. (2007) and total boron of Uppström (1974), which was used for the sake of consistency with previous studies. The CO2SYS.m script was modified to allow inputs for [Ca], [Mg], and [B] of seawater and using K1 and K2 constant sensitivities to [Mg], [Ca], and [Na] as in Ben-Yaakov and Goldhaber (1973).

2.3 Sample preparation

The calcite shells produced by foraminifers in these culture experiments were composed of chambers grown in the open ocean before collection (unconstrained, unknown conditions) and chambers grown in experimental seawater (controlled, constant conditions). For *O. universa* specimens, the contribution of juvenile chambers to the final shell weight is insignificant (Spero and Parker, 1985), and gametogenic spheres grown in culture experiments were simply cracked in half and analyzed without attempting to remove any juvenile chamber fragments. In the case of *G. sacculifer* and *G. ruber*, the contribution of open-ocean calcite to the final shell is not negligible, and was removed to enable analysis of calcite that was produced only under controlled experimental conditions. This was achieved by amputation of chambers grown during the experimental period using a medical scalpel blade under a light microscope. These amputated chambers were then weighed and pooled to make a single sample (comprising approximately 20 to 80 chambers per experiment to yield a total of 400 μ g of calcite).

Samples were cleaned according to the methods of Russell et al. (2004), using two 30-minute treatments of a hot, buffered hydrogen peroxide solution (equal parts 0.1N NaOH

+ 30% H₂O₂, 70-80°C) to remove organic matter. Finally, all cleaned samples were rinsed 3 times with ultrapure Milli-Q⁺ water that had been passed through a boron Q-gard purification filter, briefly leached with 0.001N HNO₃, and rinsed again 3 times with Milli-Q⁺ water. Immediately prior to analysis, samples were dissolved in 0.65N HNO₃ (trace element grade).

2.4 Analysis

Elemental composition of calcite samples was determined by inductively coupled plasma mass spectrometry (ICP-MS). *Orbulina universa* samples (2008 culture season) were analyzed in the Godwin Laboratory of Cambridge University (PerkinElmer SCIEX Elan DRC II), where the calcium concentration ([Ca]) of each sample solution was diluted to 100 ppm to avoid matrix effects on trace element ratios (Yu et al., 2005). *Globigerinoides* samples and *O. universa* samples from 2010 and 2013 were analyzed at Rutgers University (Thermo Scientific Element XR), where standard solutions with differing [Ca] (1.5 – 8 mM) were run to quantify and correct for any matrix effects. At Cambridge, within-run precision of an internal standard solution was <1% for all ratios analyzed; long-term precision reported as Relative Standard Deviation (RSD = [Standard Deviation/Average Value]*100) is 2.4% (Li/Ca), 1.4% (Mg/Ca), 0.9% (Mn/Ca), 0.9% (Sr/Ca), 2.4% (Cd/Ca), and 2.5% (U/Ca) (Yu et al., 2005). At Rutgers, within-run precision of three standard solutions was <3% for all ratios analyzed; long-term precision (RSD) is 5.8% (Li/Ca), 2.3% (Mg/Ca), and 4.0% (Sr/Ca). Long-term precision data were not available for Na/Ca; instead, uncertainty was estimated as the maximum difference (1.7%) between consistency standard values determined at the beginning and end of the run. Na/Ca error bars in figures represent the largest % difference among 3 consistency standards analyzed at the beginning and end of each run, or the 1σ of replicate measurements, whichever is larger.

Elemental composition of seawater from *O. universa* experiments (2013) was determined via simultaneous ICP-AES at the Australian National University (axial Varian Vista Pro). Experimental seawater was sampled before and after culturing, filtered with a 0.2μm filter, acidified with 150 μl HCl (Aristar 30%), covered with Parafilm and a screw top lid, and stored until analysis in the spring of 2014. Prior to analysis, samples were diluted 10x with 2% HNO₃ and then run using a standard-sample-standard bracketing routine with a synthetic seawater standard of known elemental concentrations. Initial and final seawater elemental compositions were averaged to determine experimental values (Table S1). Analytical precision (RSD) for seawater B/Ca, Mg/Ca, and Sr/Ca was 0.3, 0.2, and 0.2 %, respectively.

Orbulina universa produced more calcite in culture than either *G. ruber* or *G. sacculifer*, allowing a larger number of elements to be measured for *O. universa* than for other species (Table S1). *Globigerinoides ruber* or *G. sacculifer* samples were limited to the determination of Li, B, Mg, Ca, Sr, and Na. B, Mg, and Ba results are reported in Allen et al., (2012), Hönisch et al. (2013), and Hönisch et al (2011), respectively.

2.5 Assessment of correlation

To assess correlations between trace element ratios and environmental parameters, Pearson linear correlation coefficients (R) and corresponding p values were calculated. Values of $p < 0.05$ indicate > 95% confidence that the R (or R^2) value differs from zero. In this study, relationships with $p \leq 0.05$ are considered significant; a few borderline relationships ($0.05 > p < 0.1$) are discussed as well. All p values (including those > 0.05) are reported in Tables 2 (element-experimental parameter) and S1 (element-element). Exponential or other curved relationships may fail this type of significance test, so Spearman rank correlations were also evaluated to check for non-linear behavior. Results of these two tests were largely consistent and as a result the more commonly used linear Pearson coefficients are presented. Correlations were calculated using individual data points (Table S1), not experiment averages.

2.6 Estimation of net calcification rates

Net calcification rates were estimated for each cultured foraminifer by dividing the weight of calcite grown in culture by the number of growth days in culture ($\mu\text{g}/\text{day}$). For *O. universa*, the final sphere weight for each specimen was divided by the number of days that particular specimen lived between sphere formation and gametogenesis. The trochospiral shell is very small and often absent in adult shells; its contribution to the final weight was assumed to be negligible. For *G. sacculifer* and *G. ruber*, the total weight of chambers grown in culture (i.e., the amputated chambers) for each individual was divided by the total number of days that individual spent in culture. Chambers amputated from *G. bulloides* were too small to be weighed individually; their weight was typically below the detection limit of the balance. Instead, the weight of amputated chambers was estimated from the final pre-amputated shell weight (W_F) and the difference between the final shell diameter (D_F) and the amputated diameter (D_A) according to the following equation: $W_A = W_F \times (D_A - D_F)$. Finally, calcification rates determined for all specimens ($n = 551$) were used to determine averages for each experiment. The average number of specimens in each experiment was ~ 20 . Typical net calcification rates for each species were determined by averaging the results of all temperature, salinity, and pH experiments (excluding DIC experiments, which were only conducted with *G. sacculifer*).

Surface areas of cultured shells were not measured. However, to provide area-normalized estimates of calcification rates for comparison with inorganic precipitation studies (e.g., Uchikawa et al., 2015), shell surface areas were approximated according to a weight-surface area relationship for planktic foraminifera determined by the Brunauer-Emmett-Teller method (Gehlen et al., 2005). We chose the value determined from the shallowest samples in the Gehlen et al. (2005) study ($1.9 \text{ m}^2/\text{g}$) because those sediments are most likely to be well preserved and therefore most analogous to our freshly cultured samples. Rates reported in $\mu\text{g}/\text{day}$ (described above) were converted to mol/second assuming $100.1 \text{ g}/\text{mol CaCO}_3$. Finally, these values were divided by surface areas calculated from individual shell weights to estimate calcification rates in units of $\text{mol m}^{-2} \text{ s}^{-1}$.

3 Results

Net calcification rates

Different species exhibit different average calcification rates: *G. bulloides* (0.3 µg/day) < *G. ruber* (1 µg/day) < *G. sacculifer* (3 µg/day) < *O. universa* (6 µg/day) (Fig. 8). All species means are distinct from one another at a 95% confidence level. When converted to mol m⁻² s⁻¹ assuming a surface area of 1.9 m²g⁻¹, the calcification rates are: *G. bulloides* (3.0 x 10⁻⁹) < *G. sacculifer* (3.3 x 10⁻⁹) < *G. ruber* (8.6 x 10⁻⁹) < *O. universa* (1.3 x 10⁻⁸). Applying species-specific values of 1.2 and 2.0 m²g⁻¹ to the *O. universa* and *G. sacculifer* data, respectively (de Kanel and Morse, 1979), lowers the mean *O. universa* value by ~40% to 8.3 x 10⁻⁹ and raises the mean *G. sacculifer* value by ~5% to 3.5 x 10⁻⁹ mol m⁻² s⁻¹, but this does not alter the major patterns observed (Fig. S2).

Response of calcification rate (µg/day) to experimental parameters (temperature, salinity, [CO₃²⁻]) varies within and among species (Table S1). Calcification rate increases with [CO₃²⁻] in pH and DIC experiments with *G. sacculifer* (p = 0.004 and 0.003, respectively), and decreases slightly with salinity in *G. sacculifer* (p = 0.02). Other linear regressions are not significant at a 95% confidence level (p ≤ 0.05). Calcification rate in *O. universa* increases with temperature (p = 0.08) and appears to peak at pH 8.3 (total scale) (~300 µmol/kg [CO₃²⁻]) and at a salinity of ~33 (Fig. 9). Significance of trends in *G. bulloides* was not assessed due to paucity of data (n = 2 in each experiment).

Sr/Ca

Sr/Ca increased with pH in all three species: *O. universa* (R = 0.93, p = 0.02), *G. ruber* (R = 0.99, p = 0.06), and *G. sacculifer* (R = 0.83, p = 0.08) (calculated using individual points, not averages). In *G. sacculifer*, when [CO₃²⁻] is raised by increasing pH, Sr/Ca only increases by 0.016 mmol/mol per 100 µmol/kg CO₃²⁻ (R = 0.77, p = 0.12), but when [CO₃²⁻] is raised by increasing DIC and holding pH constant, Sr/Ca increases by 0.19 mmol/mol per 100 µmol/kg CO₃²⁻ (R = 0.88, p = 0.12) (Figs. 1 and 2). This strong effect of DIC manifests as more than a doubling of Sr/Ca from 0.74 to 1.6 mmol/mol with an increase in DIC from ~1150 to 4195 µmol/kg. The sensitivity of Sr/Ca to CO₃²⁻ is not constant across the experimental DIC range: below the ambient DIC of ~2000 µmol/kg, it is ~62% per 100 µmol/kg CO₃²⁻, while above ~2000 µmol/kg it drops to ~8% per 100 µmol/kg CO₃²⁻ (Fig. 2). In *O. universa*, when [CO₃²⁻] is raised by increasing DIC (holding pH constant), the Sr/Ca increase is constant across the experimental range at 0.12 mmol/mol per 100 µmol/kg CO₃²⁻ (R = 0.96, p < 0.01) (Fig. 2). Sr/Ca does not exhibit significant relationships (p < 0.05) with temperature or salinity in any single species (Table 1). When all species data are combined, there is a positive correlation with temperature (R = 0.61, p = 0.03) and salinity (R = 0.51, p = 0.06). Sr/Ca responds linearly to changes in solution Sr/Ca (Fig. 3).

Cd/Ca

In *O. universa*, Cd/Ca increases as temperature rises ($R = 0.91$, $p = 0.03$). Cd/Ca is negatively correlated with salinity, pH, and $[\text{CO}_3^{2-}]$ (Fig. 4), but the correlations are not significant ($p = 0.34$, 0.24 , and 0.48 , respectively).

Mn/Ca

In *O. universa*, Mn/Ca decreases with salinity ($R = -0.78$, $p = 0.04$), temperature ($R = -0.80$, $p = 0.10$), pH ($R = -0.95$, $p = 0.01$), and $[\text{CO}_3^{2-}]$ ($R = -0.95$, $p = 0.02$) (Figs. 1 and 4).

Mg/Ca

Mg/Ca-salinity relationships for *O. universa*, *G. ruber*, and *G. sacculifer* are reported in Hönisch et al. (2013). In *G. sacculifer*, Mg/Ca increases with DIC according to the following relationship: $\text{Mg/Ca} = \text{DIC} \cdot 1.5 \cdot 10^{-4} (\pm 1.4 \cdot 10^{-4}) + 3.5 (\pm 0.4)$ ($\pm 95\%$ confidence bounds, $n = 4$). The same Mg/Ca data regressed against $[\text{CO}_3^{2-}]$ in DIC experiments yield $\text{Mg/Ca} = [\text{CO}_3^{2-}] \cdot 1.2 \cdot 10^{-3} (\pm 1.1 \cdot 10^{-3}) + 3.5 (\pm 0.3)$. In contrast, in pH experiments with *G. sacculifer* Mg/Ca decreases slightly (~ 0.2 mmol/mol) between ~ 200 and $500 \mu\text{mol/kg}$ $[\text{CO}_3^{2-}]$. Mg/Ca decreases more strongly with $[\text{CO}_3^{2-}]$ in *G. ruber*, *O. universa*, and *G. bulloides* (Fig. 4).

In all species cultured, Mg/Ca increases with temperature (Table S1). Temperature calibrations for *O. universa* and *G. bulloides* cultured in the year 2000 were reported in Russell et al. (2004). Exponential fits of the form $\text{Mg/Ca} = B \exp(AT)$, where T is temperature, yield $B = 0.78 (\pm 0.52)$ and $A = 0.062 (\pm 0.025)$ for *G. sacculifer* ($n = 4$). With only $n = 3$, our *G. ruber* (pink) data result in uncertainties several times larger than the fit parameters themselves ($B = 1 (\pm 9)$ and $A = 0.053 (\pm 0.29)$). When combined with Mg/Ca data from Kısakürek et al. (2008) ($n = 8$ total), the fit constants for *G. ruber* are $B = 0.55 (\pm 0.33)$ and $A = 0.080 (\pm 0.022)$.

U/Ca

In *O. universa*, U/Ca decreases with $[\text{CO}_3^{2-}]$ ($R = -0.93$, $p = 0.02$), pH ($R = -0.98$, $p = 0.003$), salinity ($R = -0.74$, $p = 0.06$), and temperature ($R = -0.95$, $p = 0.01$) (Fig. 5).

Li/Ca

Li/Ca does not exhibit significant relationships with any parameters in any foraminifer species. Li/Ca of *G. ruber* (pink) in the lowest temperature experiment was anomalously high (Table S1, Fig. 6).

Na/Ca

In *G. ruber* (pink), Na/Ca increases with salinity ($\text{Na/Ca} = 0.074 (\pm 0.006) \cdot S + 4.1 (\pm 0.2)$ ($\pm 95\%$ CI); $R = 0.99$, $p = 0.01$). Correlations with temperature, salinity and $[\text{CO}_3^{2-}]$ are

not significant. In *G. sacculifer*, no significant relationships are observed (Table 1, Fig. 7).

Ba/Ca and B/Ca

Ba/Ca and B/Ca results have been reported in Hönisch et al. (2011) and Allen et al. (2012), respectively, and the primary relationships with environmental parameters are not duplicated here.

Correlations among trace elements

In the following section, only Pearson correlation coefficients (R) with p values ≤ 0.10 ($\geq 90\%$ confidence) are discussed unless otherwise noted. Data used to determine correlations are restricted to foraminifers grown in “normal” (i.e. modern) seawater; Paleocene seawater experiments conducted in 2013 are excluded from this analysis. For a full list of correlations and p values, see Table S2, and for a text description, see Section 3 of SOM.

4 Discussion

4.1 Sr/Ca

Sr/Ca ratios of foraminiferal samples grown at ambient conditions fall within the range reported by previous studies (Fig. 1) and the calculated D_{Sr} of 0.16 for our samples ($D_{Sr} = [Sr/Ca]_{calcite}/[Sr/Ca]_{seawater}$) is also consistent with previous culture experiments and core top studies ($D_{Sr} = 0.16$, Delaney and Bé, 1985; Dueñas-Bohórquez et al., 2009; Emiliani, 1955; Lea et al., 1999b; Russell et al., 2004). In addition, comparison of our modern ($Sr/Ca_{Seawater} = 8.5$ mmol/mol) and Paleocene seawater experiments ($Sr/Ca_{Seawater} = 4.5$ mmol/mol) confirms that Sr/Ca of foraminiferal calcite increases linearly with Sr/Ca of seawater ($Sr/Ca_{Foram} = 0.16 * Sr/Ca_{Seawater}$, Fig. 3).

Kısakürek et al. (2008) reported Sr/Ca increasing with salinity at a rate of 0.02 mmol/mol per salinity unit ($R^2 = 0.81$, $p = 0.10$), and Lea et al. (1999) also reported a positive Sr/Ca-salinity relationship. Single-species calibrations from our study do not yield significant relationships between Sr/Ca and salinity or Sr/Ca and temperature (Table 1). When all species data are combined, positive relationships with salinity and temperature are observed ($p = 0.03$ and 0.06 , respectively). However, minor species-specific differences in Sr incorporation could be partly responsible – *G. ruber* typically has higher Sr/Ca than *G. bulloides* at any given condition, and *G. ruber* experiments cover the upper end of the temperature range – so we do not consider these combined-species observations to provide convincing evidence of a positive Sr/Ca-temperature or Sr/Ca-salinity relationship.

A small but significant increase in Sr/Ca is observed with increasing pH. Given a glacial-interglacial surface ocean pH shift of 0.15 units (Hönisch and Hemming, 2005; Hennehan et al., 2013), the Sr/Ca shift in *G. sacculifer* would amount to a ~ 0.01 mmol/mol change.

Assuming a glacial-interglacial surface ocean shift of $\sim 100 \mu\text{mol/kg}$ $[\text{CO}_3^{2-}]$, the results from the pH experiments predict a Sr/Ca increase of $\sim 0.014 \text{ mmol/mol}$. With an analytical RSD of 4%, such changes would be undetectable.

To date, DIC experiments have only been performed with *G. sacculifer* and *O. universa*. In the DIC experiments, where $[\text{CO}_3^{2-}]$ variation is linked to DIC instead of pH, the sensitivity of Sr/Ca to $[\text{CO}_3^{2-}]$ in *G. sacculifer* is 12x higher than the sensitivity observed in pH experiments (calculated as the ratio of the $[\text{Sr/Ca}]/[\text{CO}_3^{2-}]$ slopes for DIC and pH experiments, $1.9 \mu\text{mol/mol per } \mu\text{mol/kg} / 0.16 \mu\text{mol/mol per } \mu\text{mol/kg} \approx 12$; Fig. 2 and Table S1). Why does Sr/Ca respond so differently to $[\text{CO}_3^{2-}]$ depending on whether pH or DIC is changing?

Incorporation of Sr into calcite increases with calcification rate (Gabitov and Watson, 2006; Lorens, 1981), and can be accurately simulated by diffusion-growth models in which Sr incorporation is a function of two competing processes: calcite growth rate and the rate of solution-surface molecular exchange (DePaolo, 2011). Our two different carbonate system experiments (pH and DIC) span similar calcite saturation states from $\Omega \approx 2$ to 12 (where $\Omega = ([\text{Ca}^{2+}][\text{CO}_3^{2-}])_{\text{in situ}}/[\text{Ca}^{2+}][\text{CO}_3^{2-}]_{\text{saturation}}$). If foraminiferal calcification rate simply increased with Ω , this factor alone could not explain the observed Sr/Ca behavior. On the other hand, if calcification rate is a function of Ω as well as pH and the relative proportions of Ca^{2+} and CO_3^{2-} in solution, as in inorganic experiments (Nehrke et al., 2007; Wolthers et al., 2012), this may help explain our observations. The *G. sacculifer* DIC experiment data parallel inorganic calcites grown by Nehrke et al. (2007) on a D_{Sr} vs. $[\text{CO}_3^{2-}]/[\text{Ca}^{2+}]$ plot (Fig. 10). This suggests that, as for inorganic precipitation, foraminiferal calcification rate may increase as $[\text{CO}_3^{2-}]/[\text{Ca}^{2+}]$ approaches 1, with D_{Sr} and therefore Sr/Ca increasing as calcification rate increases. Accordingly, one might speculate that the difference between our DIC and pH experiments reflects a $[\text{CO}_3^{2-}]/[\text{Ca}^{2+}]$ -driven rate influence on Sr incorporation.

Experiments with benthic foraminifera indicate that some species have the ability to vacuolize seawater and then increase its pH to values of 9 or greater, increasing the calcite saturation state and promoting calcite precipitation (Bentov et al., 2009; de Nooijer et al., 2014). Given this scenario, when foraminifers vacuolize high DIC seawater and raise the vacuole pH, they are able to drive the solution $[\text{CO}_3^{2-}]/[\text{Ca}^{2+}]$ closer to 1 and generate faster precipitation rates and greater Sr incorporation. Foraminifers could also raise the pH of low DIC seawater to the same level, but this would produce a smaller $[\text{CO}_3^{2-}]/[\text{Ca}^{2+}]$ because there is less DIC and thus less HCO_3^- available to convert to CO_3^{2-} .

Model vacuole compositions based on this thought experiment are illustrated in Fig. 10 (black triangles), along with measured Sr/Ca and $[\text{CO}_3^{2-}]/[\text{Ca}^{2+}]$ values for the experimental seawater in which foraminifera were grown (filled circles). Vacuole compositions were calculated by increasing initial experimental seawater pH to 9 (total scale) and assuming $[\text{Ca}]_{\text{sw}} = 10 \text{ mmol/kg}$ for the modern seawater experiments. Plotting observed Sr/Ca against seawater vacuole $[\text{CO}_3^{2-}]/[\text{Ca}^{2+}]$ values at pH = 9 results in alignment of the data roughly parallel to the inorganic calcite Sr/Ca- $[\text{CO}_3^{2-}]/[\text{Ca}^{2+}]$

relationship observed by Nehrke et al. (2007), which is consistent with the idea that calcification rate is influenced by $[\text{CO}_3^{2-}]/[\text{Ca}^{2+}]$ and Sr/Ca increases with calcification rate.

Rates of calcification are notoriously difficult to measure in foraminifera. In this study, we provide approximations of net average calcification rates for each experiment (Table S1). Average *G. sacculifer* calcification rates from the pH and DIC experiments increase consistently as seawater $[\text{CO}_3^{2-}]$ increases (Fig. 9), which supports the rate-controlled Sr incorporation scenario posited above. However, D_{Sr} values do not increase consistently with average calcification rates (Fig. 11). Potential explanations for this discrepancy include: 1) If foraminifera calcify episodically (i.e., in short bursts of growth), then our net calcification rates, which were calculated assuming continuous growth, do not accurately represent the true, instantaneous calcification rates, or 2) D_{Sr} is not rate-controlled. In the latter case, it is possible that another factor is at work, perhaps variation in the availability of free Sr or Ca ions. Calculation of dissolved metal speciation is beyond the scope of the present study, but dissolved ion speciation and availability may vary with the presence of certain organic ligands and with the major composition of seawater (Stumm and Morgan, 1996). At the moment, we can not yet definitively confirm or rule out a calcification rate effect on Sr/Ca with the available data.

It is interesting to note that, with the exception of the lowest-DIC experiment, *O. universa* Sr/Ca results also converge on the same trend-line as *G. sacculifer* when the above model is applied (internal pH = 9, Fig. 10, gray diamonds). The two sets of DIC experiments (*G. sacculifer* and *O. universa*) have similar slopes but are offset from each other when plotted against $[\text{CO}_3^{2-}]/[\text{Ca}^{2+}]$ (Fig. 10). This offset could be due to: 1) lower average pH of *O. universa* experiments, perhaps leading to greater HCO_3^- involvement in calcification, causing rates to be maximized at lower solution $[\text{CO}_3^{2-}]/[\text{Ca}^{2+}]$ ratios (as observed for inorganic calcites by Wolthers et al. (2012), 2) higher average calcification rates in *O. universa* experiments (Fig. 8) leading to greater Sr incorporation than in *G. sacculifer*, or 3) differences in major element composition of experimental seawater between studies (Catalina 2013 experiments with *O. universa* were conducted in model Paleocene seawater with $\text{Mg} \approx 30$ mmol/kg and $\text{Ca} \approx 20$ mmol/kg, whereas Puerto Rico 2010 experiments with *G. sacculifer* used ambient seawater, with $\text{Mg} \approx 50$ mmol/kg and $\text{Ca} \approx 10$ mmol/kg).

The behavior of Sr/Ca in the constant-pH, variable-DIC experiments is intriguing, and if other proxies can be used to estimate another carbonate system variable (e.g., $\delta^{11}\text{B}$ for pH), Sr/Ca might prove useful for constraining the seawater carbonate system. Given the possibility of a rate influence on Sr/Ca partitioning, it will be important to gather additional calcification rate data (preferably with higher temporal resolution, e.g., hourly measurements) and to evaluate the extent of other, independent influences on calcification rate such as light and food availability.

4.2 Mn/Ca and Cd/Ca

Mn and Cd may be influenced by similar mechanisms during calcite growth as they share the same ionic charge (+2), have similar effective ionic radii, are highly compatible ($D > 1$), and both substitute for Ca in the calcite lattice (Lorens, 1981; Reeder, 1996; Wartel et al., 1991). Experiments with inorganic calcite have revealed decreasing Mn/Ca and Cd/Ca with increasing calcite growth rate (Lorens, 1981). If foraminiferal calcification rates increase as pH and $[\text{CO}_3^{2-}]$ increase, then the more energetically-demanding dehydration and incorporation of smaller ions might be expected to increasingly exclude Mn and Cd during more rapid calcite growth (DePaolo, 2011). Another possible kinetic mechanism is described by an alternative growth entrapment model in which element incorporation (or entrapment) in the bulk crystal lattice depends on the competing factors of growth rate and ion *diffusivity* within the mineral surface layer (Watson, 2004). Either of these kinetic mechanisms could play a role in our observed decreases in Mn and Cd incorporation at higher pH and $[\text{CO}_3^{2-}]$ (Fig. 4).

In culture experiments, *G. bulloides* consistently has higher Mn/Ca and Cd/Ca than *O. universa*. Mashiotta et al. (1997) offered two possible explanations for the Cd/Ca difference: 1) *G. bulloides* calcifies more slowly than *O. universa* due to its lack of symbionts and correspondingly lower-saturation microenvironment, leading to higher Cd/Ca for kinetic reasons (discussed above) or 2) the dinoflagellate symbionts borne by *O. universa* remove Cd directly from solution during photosynthesis, lowering the concentration of Cd in the microenvironment and leading to lower Cd/Ca of the shell. If symbionts also remove Mn from the microenvironment, this might explain the consistent Mn/Ca and Cd/Ca offset between species. It is not known whether foraminifera or their symbionts actively take up Mn, but in some algae Mn is used as a cofactor in metalloenzymes (Sunda, 1989).

Average net calcification rates do increase with $[\text{CO}_3^{2-}]$ in both *G. bulloides* and *O. universa* experiments (Fig. 9), consistent with a rate control. Calcification rates in *O. universa* experiments are also 5-6 $\mu\text{g/day}$ higher than *G. bulloides* experiments on average, providing additional support for the idea that higher calcification rates lead to lower Mn/Ca and Cd/Ca. However, it appears that there may be a calcification rate maximum at $\sim 300 \mu\text{mol/kg } [\text{CO}_3^{2-}]$ in *O. universa*, which is inconsistent with a calcification rate control on the continuous decline in Mn and Cd incorporation. This possibility is difficult to assess since *O. universa* calcification rates are so variable (see 1σ error bars in Fig. 8). Symbiont activity could well be an additional or alternative control. Low light experiments at high saturation state (high $[\text{CO}_3^{2-}]$ and/or $[\text{Ca}^{2+}]$) might allow us to disentangle the two parameters, if calcification rates can be closely monitored.

The influence of temperature on Cd and Mn incorporation in calcite can be predicted from thermodynamic principles, assuming equilibrium conditions and applying the Van't Hoff equation to the equilibrium constants (Haynes, 2014). Such calculations predict a slight decrease in Cd/Ca and Mn/Ca with temperature (Fig. S1). However, in *O. universa*, Mn/Ca decreases with temperature ($R=-0.8$, $p=0.10$) while Cd/Ca increases with temperature ($R=0.91$, $p=0.03$), defying theoretical predictions. Additional discussion of theoretical vs. observed partitioning is presented in section 4.8.

4.3 Mg/Ca

The use of Mg/Ca as a temperature proxy has been extensively discussed in the literature, and a portion of our results have already been published in Hönisch et al. (2013). Our temperature results are broadly consistent with previous calibrations and thus increase confidence in existing relationships. For this comparison we only consider data from studies that employed a strong oxidation step in the cleaning procedure (H_2O_2 of 30% or greater), since this step results in Mg/Ca values that can differ significantly from previous techniques with weaker oxidation (Russell et al., 2004). Our *G. sacculifer* temperature sensitivity (7.8%) is closer to the “with sac” sediment-trap calibration of Anand et al. (2003) than the “without-sac” calibration (6.9% versus 4.8% increase in Mg/Ca per °C, respectively). In our high-pH experiment, several final “sac” chambers were amputated and analyzed separately ($n=12$), yielding a lower Mg/Ca value than non-sac chambers (3.3 ± 0.1 and 3.6 ± 0.1 mmol/mol, respectively). It is possible that differences in growth habit or mechanism between sac and non-sac chambers may explain some of the difference between the with- and without-sac calibrations.

Data from two of our three *G. ruber* (pink) Mg/Ca temperature experiments overlap with the core-top calibration of Anand et al. (2003) within analytical error. The exponential fit parameters for the *G. ruber* (pink) culture data have wide 95% confidence envelopes, encompassing all previous core-top and culture calibrations with *G. ruber* (white) (Kısakürek et al., 2008). *Orbulina universa* Mg/Ca data are consistent with the calibration of Russell et al. (2004), which has a higher pre-exponential constant (higher Mg/Ca values, on average) than the Sargasso Sea sediment trap study by Anand et al. (2003). In short, results from our culture experiments are consistent with earlier sediment trap and culture Mg/Ca-temperature calibrations (Figs. 1 and 4), with the exception of the *O. universa* sediment trap calibration (Anand et al., 2003). The influence of salinity on Mg/Ca is minor compared to that of temperature; recommended Mg/Ca-salinity calibrations can be found in Hönisch et al. (2013).

In pH experiments, Mg/Ca of *O. universa* and *G. bulloides* increases with decreasing $[\text{CO}_3^{2-}]$ below a threshold of $\sim 200 \mu\text{mol/kg}$ $[\text{CO}_3^{2-}]$ (Fig. 4), largely consistent with previous work (Russell et al., 2004). The Mg/Ca value from our highest-pH experiment with *O. universa* ($[\text{CO}_3^{2-}] = 581 \mu\text{mol/kg}$) falls lower than all other values at lower $[\text{CO}_3^{2-}]$, hinting at non-linear behavior, perhaps a second inflection point or a continuous linear response of Mg/Ca to $[\text{CO}_3^{2-}]$ as suggested by Evans et al. (2016). Mg/Ca of *G. ruber* (pink) is also higher at lower $[\text{CO}_3^{2-}]$, similar to the *G. ruber* (white) results of Kısakürek et al. (2008), though the trend in *G. ruber* (pink) is not significant at a 95% confidence level and neither is the trend in *G. sacculifer* (Table 1). A calcification rate control on Mg incorporation is one possible explanation for the species that do exhibit an Mg/Ca response to the carbonate system, perhaps with faster calcification rates at higher $[\text{CO}_3^{2-}]$ leading to greater Mg exclusion. Such a trend could be explained by either of the growth entrapment models discussed above in 4.2 (Watson, 2004; DePaolo, 2011). Net calcification rates for *O. universa* support the idea of a rate control on Mg incorporation, with rates increasing rapidly until $\sim 200\text{-}300 \mu\text{mol/kg}$ and then leveling out (Fig. 9).

However, calcification rate data from other species do not increase detectably with $[\text{CO}_3^{2-}]$ and/or do not exhibit any kind of threshold behavior. Also, contrary to expectations described above, the fast-growing *O. universa* is characterized by higher Mg/Ca than the slow-growing *G. bulloides*, on average. An additional complication for the $[\text{CO}_3^{2-}]$ -driven rate control suggested above is the discrepancy between Mg/Ca- $[\text{CO}_3^{2-}]$ relationships determined in pH and DIC experiments, discussed below.

In *G. sacculifer*, Mg/Ca increases with DIC (and also $[\text{CO}_2]$, $[\text{CO}_3^{2-}]$ and $[\text{HCO}_3^{2-}]$) in constant-pH, variable-DIC experiments (Table 1, Fig. 12). In these experiments, Mg/Ca increases with $[\text{CO}_3^{2-}]$, in contrast to the insignificant response to $[\text{CO}_3^{2-}]$ observed in pH experiments (Fig. 4). This contrasting behavior suggests that $[\text{CO}_3^{2-}]$ (or pH) can not be the only carbonate system parameter that exerts control on Mg/Ca. In Figure 12B, the highest pH and highest DIC experiments are characterized by similar $[\text{CO}_3^{2-}]$ values ($\sim 500 \mu\text{mol/kg}$) but very different Mg/Ca values (3.6 and 4.1 mmol/mol, respectively). These results indicate that neither $[\text{CO}_3^{2-}]$, DIC, nor calcification rate can consistently explain all of the *G. sacculifer* Mg/Ca data.

What kind of effect might this have on *G. sacculifer* paleo-temperature records, if DIC is indeed influencing Mg/Ca? Applying our calibrations to estimated changes across Pleistocene ice age cycles (calculated assuming glacial-interglacial differences of -4°C , +1 salinity unit, $+100 \mu\text{mol/kg } [\text{CO}_3^{2-}]$ or $+100 \mu\text{mol/kg DIC}$), the predicted influence of DIC is $\sim 0.015 \text{ mmol/mol}$, which is very small compared to the temperature effect of 0.8 mmol/mol . The predicted impact of salinity (0.2 mmol/mol) is $\sim 10\times$ larger than that of DIC, and has more potential to impact paleo-temperature estimates (e.g., Khider et al., 2014). Altogether, our calibrations predict a glacial-interglacial Mg/Ca change of 0.6 mmol/mol if salinity and DIC effects are taken into account. Does $[\text{CO}_3^{2-}]$ influence Mg/Ca observed in pH experiments? Some previous work indicates that the carbonate ion effect is only active below $\sim 200 \mu\text{mol/kg } [\text{CO}_3^{2-}]$ (Russell et al., 2004), while other results suggest that the effect may be felt across a much wider $[\text{CO}_3^{2-}]$ range (Evans et al., 2016). More experiments are needed to resolve specific controls (DIC vs. pH vs $[\text{CO}_3^{2-}]$) and the shape of their Mg/Ca response (linear, nonlinear, etc.).

In summary, any carbonate system effects on Mg/Ca in *G. sacculifer* are expected to be within analytical error for most late Pleistocene records. The influence of DIC would constitute $\sim 2\%$ of the Mg/Ca signal across a 4°C temperature change according to the *G. sacculifer* Mg/Ca-DIC relationship (reported in Section 3). An exception might be in ocean regions where DIC-rich waters upwell to the surface. In deeper-dwelling or high-latitude species, the effect of carbonate system changes may be larger relative to temperature effects on Mg/Ca than in the species discussed here, particularly if the habitat conditions fall at the lower end of the exponential Mg/Ca-temperature calibrations and $[\text{CO}_3^{2-}]$, DIC, or pH variations are large. Ultimately, the observation that no single carbonate system parameter appears to stand as a dominant control on Mg/Ca in culture experiments leads us to recommend that carbonate system corrections not be applied to planktonic Mg/Ca records until the specific control(s) have been identified through additional culture experiments and/or field studies.

4.4 U/Ca

U/Ca in *O. universa* from our carbonate system experiments agrees well with the results of Russell et al. (2004), and yields a combined U/Ca sensitivity of $-28 \pm 10\%$ per 100 $\mu\text{mol/kg CO}_3^{2-}$ ($R^2 = 0.9$, Fig. 5). We further observe a decrease in U/Ca with temperature in *O. universa* of 0.51 ± 0.32 nmol/mol per $^\circ\text{C}$ ($R^2 = 0.9$, Fig. 5), whereas no significant temperature effect was observed by Russell et al. (2004). A U/Ca temperature decrease is predicted from thermodynamics, assuming incorporation proceeds according to the exchange reaction $\text{UO}_2^{2+} + \text{CaCO}_3 = \text{Ca}^{2+} + \text{UO}_2\text{CO}_3$ (Russell et al., 2004). However, K_{eq} calculated in this way is 1,000 times greater than observed D_{U} in foraminifera, and the theoretical rate of decrease per $^\circ\text{C}$ is also orders of magnitude higher than observed (Fig. S1). Possible reasons for the difference between this prediction and our observations are discussed in section 4.8.

Our results confirm a relationship between U/Ca and carbonate ion. The disagreement that exists between culture experiments and core-top calibrations regarding the sensitivity of U/Ca to temperature requires further investigation.

4.5 Li/Ca

Li/Ca offsets between species observed in the present study indicate at least some level of biological control on the uptake of Li from seawater (Li/Ca in *G. ruber* > *G. sacculifer* > *O. universa*) (Fig. 6), consistent with previous work that showed species-specific Li isotope and Li/Ca offsets from seawater in foraminifera from core-tops (Hathorne and James, 2006). In addition, the average partitioning of Li into foraminiferal calcite in this study, $D_{\text{Li}} = 0.006$, is higher than inorganic calcite D values, which range between ~ 0.001 to 0.002 (Okumura and Kitano, 1986; Marriott et al., 2004).

No significant change in Li/Ca was observed in *G. ruber* or *G. sacculifer* across a temperature range from 24 to 29 $^\circ\text{C}$, nor in *O. universa* from 18 to 27 $^\circ\text{C}$ (Fig. 6). This is consistent with core-top studies that have found no relationship between temperature and foraminiferal Li/Ca (Delaney and Bé, 1985; Hall and Chan, 2004), but conflicts with benthic foraminifera data that show Li/Ca decreasing with temperature (Marriott et al., 2004) and with a theoretical prediction that Li/Ca should increase with temperature (Hall and Chan, 2004). One high Li/Ca value stands out in our results – the low-temperature *G. ruber* experiment (24 $^\circ\text{C}$). It is not clear why this value was high; there were no indications of contamination. There is not sufficient evidence to interpret this value in terms of a temperature effect, but this observation warrants further investigation through experiments and field studies.

In inorganic calcites, Li/Ca increases four-fold between salinities of 10 and 50 (Marriott et al., 2004), such that a $\sim 30\%$ increase in foraminiferal Li/Ca might be anticipated between $S=30$ and 40, which is $\sim 5\times$ greater than analytical uncertainty. However, salinity shows no significant influence on the Li/Ca composition of cultured foraminifera across this range.

Hall and Chan (2004) proposed that a $[\text{CO}_3^{2-}]$ -driven calcification rate effect might explain observations of elevated Li/Ca in planktic foraminifera during glacial periods, but in our culture experiments, Li/Ca does not change significantly across a range of ~70 to 600 $\mu\text{mol/kg CO}_3^{2-}$. Moreover we have cultured the same foraminifer species as studied by Hall and Chan (2004) in core-top sediments, and our $[\text{CO}_3^{2-}]$ experiments span over 400 $\mu\text{mol/kg}$. This is a wider range wider than any glacial-interglacial $[\text{CO}_3^{2-}]$ change reconstructed to date. No significant Li/Ca response to DIC was observed in our experiments, either (Table 1). Nonetheless elevated Li/Ca in glacial samples has been documented in several species, and at other sites as well (e.g., Burton and Vance, 2000; Ni, 2010), so this is a robust observation. The lack of a carbonate ion response in our experimental data highlights the need to identify the cause for elevated glacial Li/Ca records.

4.6 Na/Ca

Partitioning of Na into foraminiferal calcite ($D_{\text{Na}} = 0.0001$, average of ambient *G. ruber* and *G. sacculifer* samples) is lower than partitioning measured for inorganic calcite ($D_{\text{Na}} = 0.0003$ to 0.0008 , Busenberg and Plummer, 1985; White, 1978), suggesting a biological control and/or differing calcification rates between these foraminiferal and inorganic calcites. Although there is robust evidence for direct substitution of divalent cations into the Ca^{2+} site in calcite, the location and mechanism of univalent (+1) cation incorporation is much less clear. Similar to Li^+ , available inorganic data suggest Na^+ may be incorporated primarily at interstitial sites in the lattice (Ishikawa and Ichikuni, 1984), and observed increases in Na/Ca with calcite growth rate may stem from a greater proportion of crystal defects leading to greater interstitial incorporation (Busenberg and Plummer, 1985). It is possible that this kind of mechanism influences Na incorporation in foraminiferal calcite as well, though there is no clear relationship between D_{Na} and net calcification rate in *G. ruber* or *G. sacculifer* (Fig. 11). There does appear to be a pattern of impurity partitioning among species. For example, *G. ruber* consistently has the highest concentrations of B, Li, and Na (all non-Ca substituting elements) of all species cultured. It would be interesting to assess whether the nature of *G. ruber* calcite is more conducive to impurity inclusion due to a greater occurrence of lattice imperfections or some other difference in calcite structure.

In our culture experiments, Na/Ca of *G. ruber* increases by ~1% per salinity unit ($R = 0.99$, $p = 0.01$), but the *G. sacculifer* data are scattered ($R = 0.14$, $p = 0.86$) (Fig. 7). The slope of the *G. ruber* Na/Ca-salinity linear regression (0.074 mmol/mol per salinity unit) is less than half the value determined for the benthic foraminifer *Ammonia tepida* (0.22 mmol/mol per salinity unit; Wit et al., 2013). Considering the current paucity of calibration points ($n = 3$ for *G. ruber*, $n = 4$ for *G. sacculifer*), our calibrations should be treated with caution. According to the *G. ruber* calibration, an increase of 1 salinity unit should result in a Na/Ca increase of ~0.1 mmol/mol. This increase, roughly the mean change predicted for the last glacial maximum, is within the estimated analytical uncertainty for Na/Ca (1.7% of 6 mmol/mol equals 0.1 mmol/mol Na/Ca). In addition, the Na/Ca relationships with S, T, and pH observed in culture all predict a Na enrichment during glacial periods; these effects will be difficult to disentangle.

4.7 The influence of calcification rate

Variation in calcification rate has been called upon previously to explain trace element behavior in some biogenic calcites (e.g., Elderfield et al., 1996; Ni et al., 2007). Simple kinetic models have also been used recently to describe observed variation in Sr/Ca and Mn/Ca partitioning in inorganic calcite as a function of calcification rate (DePaolo, 2011; Watson, 2004). In these models, kinetic fractionation operates by “growth entrapment” of impurity ions (such as Sr). The effectiveness of this entrapment varies with crystal growth rate. Different mechanisms have been proposed, including variable rates of ion hydration and dehydration at the calcite surface (DePaolo, 2011) and competition between mineral precipitation and ion diffusion within the crystal lattice (Watson, 2004). The results of inorganic precipitation experiments appear consistent with predictions based on these growth entrapment models – with increasing precipitation rate, the incorporation of Sr^{2+} , Ba^{2+} , and Na^{+} (larger than Ca^{2+}) increases, and the incorporation of Cd^{2+} and Mn^{2+} (smaller than Ca^{2+}) decreases (Fig. 13).

In principle, we should be able to evaluate the hypothesis of a rate effect in foraminiferal calcite using our trace element results alone. Given some variation in the rate of calcification, the growth-entrapment model predicts: 1) positive correlations between Cd/Ca and Mn/Ca (small cations) as well as between Sr/Ca and Ba/Ca (large cations) and 2) inverse correlations between the small and large cations (i.e., Sr/Ca and Mn/Ca should be inversely correlated). In addition, recent inorganic experiments suggest that B/Ca increases with growth rate (Uchikawa et al., 2015), so if this kinetic effect applies to foraminifera, we should also see positive correlations between the large cations and B/Ca. If U is adsorbed at the calcite surface as UO_2^{2+} , it might be expected to behave like a large cation as well, i.e., its incorporation increasing with growth rate.

Our trace element results provide mixed support for these predictions (Table S2). The most consistent and significant relationships are: 1) the inverse correlation between U/Ca and B/Ca and 2) the positive correlation between U/Ca and Mn/Ca. These correlations are observed in all *O. universa* experiments (temperature, salinity, and pH), and are characterized by R values > 0.9 and p values < 0.05. Both of these prevalent correlations (U/Ca-B/Ca and U/Ca-Mn/Ca) are opposite in sign to what is predicted based on growth entrapment. In *O. universa* (the only species for which Mn, Cd, and U were measured), Sr/Ca is inversely correlated with Cd/Ca and Mn/Ca in some experiments (consistent with growth entrapment), but not others. For example, Sr/Ca and Cd/Ca are inversely correlated in the salinity experiment ($R = -0.70$, $p = 0.08$), as predicted, but the correlation is not significant in the pH experiment ($R = -0.56$, $p = 0.33$). Mn/Ca and Cd/Ca are inversely correlated in temperature and salinity experiments ($R = -0.93$, $p = 0.02$ and $R = -0.22$, $p = 0.64$), contrary to expectations. Ba/Ca does not exhibit significant relationships with Sr/Ca, Cd/Ca or Mn/Ca. This is not unexpected, considering that Ba/Ca is roughly constant across all temperature, salinity, and pH experiments (Hönisch et al., 2011). If symbionts preferentially remove Cd and/or Mn from the micro-environment, as suggested in the discussion above, it is possible that the expected kinetic effects are not observed because they are overprinted by symbiont activity. It is not clear

why Sr/Ca and Ba/Ca, which have similar properties, behave differently. In summary, the suite of trace element data does not consistently follow predictions based on growth entrapment models, indicating that calcification rates (at least according to these models' predictions) do not dominate trace element incorporation in foraminifera. However, these results do not exclude a potential role for a minor (or even major) calcification rate influence for all species or under all circumstances.

Net calcification rates estimated from our culture experiments provide additional insight into the question of growth rate control on trace element incorporation. It is important to keep in mind that because we calculated calcification rates based on final post-gametogenic shell weights and total growth periods, our calcification rate data represent *average* rates. A value of 5 $\mu\text{g/day}$ could be produced by slow, continuous addition of calcite at a constant rate of 5 $\mu\text{g/day}$, or it could be produced by short periods of very rapid calcite growth ($\gg 5 \mu\text{g/day}$) separated by periods of very slow growth ($\ll 5 \mu\text{g/day}$) or by periods of no growth at all. There is some geochemical evidence for variation in calcification rate during the life span of *O. universa*, with faster growth during the day, perhaps peaking a few hours after first light, and slower growth at night (Spero and Parker, 1985; Spero, 1988; Lea et al., 1995; Eggins et al., 2004; Spero et al., 2015). Therefore values discussed below do not necessarily represent instantaneous calcification rates, but they do provide useful estimates of net average calcification rates for comparison with the bulk geochemical data. Our average *O. universa* calcification rates ($6 \pm 1 \mu\text{g/day}$) overlap with a previous estimate of $\sim 7 \mu\text{g/day}$ based on uptake of the radionuclide $^{45}\text{Ca}^{2+}$ in high-light experiments (Lea et al., 1995).

The most striking features of the calcification rate dataset are the offsets between species (Fig. 8). Net calcification rates are highest in *O. universa* and lowest in *G. bulloides*. The observation of consistently higher rates in symbiont-bearing species compared to the symbiont-barren species (*G. bulloides*) is consistent with the idea that photosynthetic symbiont activity enhances calcification rates (Bé et al., 1992; Lea et al., 1995; Caron et al., 1982). Calcification rate differences among the three symbiont-bearing species might be related to differences in the number and/or photosynthetic activity of symbionts typically associated with foraminifers of each species type. This could be tested in future culture studies by counting the number of symbionts per host and/or quantifying photosynthesis rates (e.g., Spero and Parker, 1985).

Calcification rates do not respond uniformly to changes in experimental conditions (Figs. 8 and 9). The only significant correlations are *G. sacculifer* calcification rate with salinity ($p = 0.02$) and *G. sacculifer* calcification rate with $[\text{CO}_3^{2-}]$ in both the pH and DIC experiments ($p = 0.004$ and 0.003 , respectively). Calcification rate in *O. universa* exhibits a weak correlation with temperature ($p = 0.08$). The lack of a consistent response to external factors such as temperature, salinity, pH, and $[\text{CO}_3^{2-}]$ in all (or even more than one) species suggests that foraminifera are not strongly affected by these external factors. In contrast, in inorganic calcite precipitation experiments (Uchikawa et al., 2015), calcite calcification rates increase by an order of magnitude over an experimental $[\text{CO}_3^{2-}]$ range similar to ours. The difference between the rapid response of inorganic calcite calcification rates and the undetectable or variable response of foraminiferal calcite

calcification rates to $[\text{CO}_3^{2-}]$ suggests that foraminifera exert strong control over calcification rate. This makes sense given the intricate architecture of foraminiferal calcite and considering the fact that having the power to control calcification rates would aid survival (e.g., runaway calcification could lead to sinking out of the photic zone and likely mortality). A biologic control on calcification rate could also help explain the inter-species differences discussed above.

When combined with the geochemical data, the calcification rates provide new insight into some of the questions raised earlier. Based on growth entrapment model predictions, the slow-growing *G. bulloides* should have higher Cd/Ca and Mn/Ca than fast-growing *O. universa*, which it does (Fig. 4). Based on the same premise, *O. universa* should have higher Sr/Ca and Ba/Ca than *G. bulloides*, however, there is no discernible difference between these species' average values for Sr/Ca and Ba/Ca (Figs. 1 and 2; see also Hönisch et al., 2011). So, as in the above discussion of geochemical data, support for calcification rate effects is mixed.

When all partition coefficients (D values) for all experiments are plotted against net calcification rates for each species, a few broad patterns emerge (Fig. 11). With the exception of *G. bulloides*, D_{Li} , D_{B} , and D_{U} decrease with increasing calcification rate, while D_{Mg} increases. D_{Sr} , D_{Ba} , D_{Mn} , D_{Cd} , and D_{Na} exhibit no clear relationship to calcification rate. Na/Ca has been observed to increase with calcification rate in inorganic experiments (Mucci, 1988), yet there is no consistent or significant relationship between net calcification rate and D_{Na} in *G. ruber* and *G. sacculifer* (Fig. 11), suggesting that calcification rate is not the dominant control on Na incorporation in planktic foraminifers under these specific experimental conditions. The Mg ion is similar to Cd and Mn ions (same charge, similar radius), so it might be expected to behave in a similar manner. However, the increase of D_{Mg} with calcification rate opposes the decline predicted for Cd and Mn incorporation, and D_{Mn} and D_{Cd} are scattered (Fig. 11).

Boron partitioning increases exponentially with growth rate (precipitation rate) in experiments with inorganic calcite (Uchikawa et al., 2015). The fit to experimental data is optimized when boron incorporation is expressed in terms of a new formulation of the empirical partition coefficient: $\lambda_{\text{BT/DIC}} = [\text{B/Ca}]/[\text{B}_\text{T}/\text{DIC}]$, where $\text{B}_\text{T} = [\text{B}(\text{OH})_4^-] + [\text{B}(\text{OH})_3]$. This formulation implies that both boric acid and borate are incorporated into calcite. Uchikawa et al. (2015) speculated that boric acid incorporation might increase with growth rate in inorganic calcite. An influence of calcification rate on B incorporation in planktic foraminifers was also suggested by Ni et al. (2007) based on an observed increase in B/Ca with shell size in core-top samples. In our *G. sacculifer* culture experiments, B/Ca increases with calcification rate in pH experiments, but it decreases with calcification rate in DIC experiments (Fig. 14A). When data from all species are combined, the relationship between calcification rate and boron incorporation (expressed as D_{B}) is variable (Fig. 14B). If anything, D_{B} appears to decrease as calcification rate increases, as does the partition coefficient suggested by Uchikawa et al. (Fig. 14C). The contrast between trends in culture and inorganic experiments suggests that the rate-dependent mechanism driving B incorporation in inorganic calcites (e.g., increasing boric acid incorporation at higher calcification rates) is not active during foraminiferal

calcification, or that it is overwhelmed by other processes. Net calcification rates for foraminifers in our experiments are ~100x slower than the slowest rates observed by Uchikawa et al. (2015), so it may be that any rate influence on B incorporation is greatly diminished or negligible at very slow growth rates, as suggested by those authors. Thus although our results do not rule out a role for calcification rates, the available data do not support a detectable influence of calcification rate on B/Ca in planktonic foraminifera.

4.8 Theoretical predictions, inorganic calcite, and foraminiferal calcite

We calculated equilibrium partition coefficients for many trace elements from the Gibbs free energies of ideal products and reactants. Implicit in these thermodynamics-based calculations are several assumptions, including: 1) the system is in equilibrium, 2) the activities of all chemical species equal 1 in both solution and solid phases, and 3) substances are pure and ideal (no interactions among impurities, etc.). None of these assumptions hold in the formation of foraminiferal calcite, because net growth requires some level of disequilibrium, activities of most ions in seawater are not equal to 1, and solutions and solids are a mixture of various components, including some organic matter. Despite the fact that these theoretical predictions are not likely to predict trace element behavior in foraminiferal calcite, considering the size and direction of different offsets between empirical distribution coefficients and their theoretical counterparts may still provide insight into trace metal incorporation.

By comparing experimental distribution coefficients (i.e., D values) with theoretical predictions (K_{eq} values), Rimstidt et al. (1998) found evidence for a kinetic influence on the incorporation of cations in inorganic calcite, including Ba^{2+} , Cd^{2+} , Cu^{2+} , Fe^{2+} , Mg^{2+} , Mn^{2+} , Sr^{2+} , and UO_2^{2+} . Those authors also observed a consistently larger offset between theoretical and empirical partition coefficients for cations larger than Ca^{2+} (e.g., Sr^{2+} , Ra^{2+} , Ba^{2+}) than for ions smaller than Ca^{2+} . Rimstidt et al. (1998) suggested that the larger offset might be due to the fact that large ions do not fit as easily into the calcite lattice as smaller ions, and that partitioning is optimized as ions approach the ionic radius of Ca^{2+} . The D values for inorganic calcite and foraminiferal calcite (Fig. 13) appear largely consistent with these ideas. Foraminiferal D values increase with increasing ionic radius from Mg to Li to Mn (Cd is an exception, discussed below), and the larger cations Sr^{2+} and Ba^{2+} are characterized by smaller D than Mn and Cd.

For some elements like Ba, Sr, Na, and Mn, the partitioning into foraminifera and inorganic calcites is similar, indicated by roughly equivalent D values (Fig. 13). This suggests that a simple combination of thermodynamic and kinetic factors may reasonably predict element behavior, as in Rimstidt et al. (1998). In contrast, some elements are not easily explained by thermodynamics and kinetics alone. Some offsets between inorganic and foraminiferal D values suggest biological control on the composition of calcifying fluids and/or biogenic calcite, or perhaps activity coefficients that significantly differ from 1. For example, the partition coefficient for Cd/Ca in *O. universa* (D_{Cd}^{foram}) is orders of magnitude smaller than both the inorganic and theoretical partition coefficients. This could be caused by selective uptake of Cd by symbionts, as proposed by Mashiotto et al. (1997). Partitioning of Mg/Ca is also lower in foraminifera than in inorganic calcites (Lea

et al., 1999; Russell et al., 2004), which might be due to selective ion pumping exercised to make calcite precipitation more energetically favorable (Zeebe and Sanyal, 2002).

For the singly charged cations Li^+ and Na^+ , theoretical predictions are several orders of magnitude lower than observations (Fig. 13). This is likely due to multiple factors, one of which is that the calculation was performed assuming the substitution reaction $2\text{Li}^{2+} + \text{CaCO}_3 = \text{Li}_2\text{CO}_3 + \text{Ca}^{2+}$ (same for Na^+). If these ions are incorporated in interstitial positions, as discussed above, and/or they are not competing with Ca^{2+} for lattice sites (Langer et al., 2015), then this equation would not be applicable to inorganic or foraminiferal calcite samples.

4.9 Calcification mechanisms

To understand foraminiferal calcite composition, we must not only consider fundamental inorganic trace element partitioning and kinetic effects, but also biological modification of calcifying fluid composition. Living organisms employ many strategies to induce and control calcite growth, and in foraminifera these strategies appear to focus on the isolation and modification of seawater (e.g., Bé and Hemleben, 1970; Bentov et al., 2009; Erez, 2003; Nehrke et al., 2013). The manipulation of barriers to nucleation and growth through addition or removal of key compounds seems to play an important role as well. Many mechanisms have been proposed, including seawater vacuolization, pumping of Ca^{2+} , Mg^{2+} , H^+ , or HCO_3^- , carbon concentration via diffusion of CO_2 across a concentration gradient, formation and delivery of amorphous calcium carbonate to sites of calcification, and others, though direct observations of most internal processes are limited (de Nooijer et al., 2014; Elderfield et al., 1996; Erez, 2003; Zeebe and Sanyal, 2002).

One of the models first proposed to explain trace element incorporation in foraminifera is based on the assumption that seawater is vacuolized, and then only a fraction (f) of this internal pool of ions is utilized for calcification (Elderfield et al., 1996). To assess whether the present culture data are consistent with such a model, we calculated empirical partition coefficients using inorganic partition coefficients (α) via a Rayleigh distillation equation ($D_{\text{Tr}} = (1 - f^\alpha)/(1 - f)$). If the model's assumptions are correct, each observed foraminiferal partition coefficient (D_{foram}) should yield a utilization fraction f that is consistent with estimates from other trace elements. However, this is not the case – according to the Rayleigh calculations, Mg, Zn, Na, and U results imply a >99% utilization of the reservoir while Ba and Li imply < 1% utilization. Both can not be correct. This outcome does not preclude the possibility that variable fractions of an internal seawater reservoir are used during calcification, but it does indicate that other processes involving selective transport and/or selective incorporation or discrimination of different elements are at work.

4.10 Searching for a useful conceptual framework

Although many questions remain unanswered, we know all calcification mechanisms are unified by a common goal: to combine dissolved components into a solid shell. To

achieve this, foraminifera must manipulate the “energy landscape” between solution and solid. This concept is described by De Yoreo and Vekilov (2003): “... crystallization is, first and foremost, a phase transition through which matter is transformed from a state of high free energy in a solvated state to one of low free energy in the crystal lattice. All aspects of a crystal, including its phase, habit, and growth rate, are determined by the shape of the landscape.” To fully understand trace element partitioning between seawater and foraminiferal calcite, we will need to consider the underlying inorganic controls and biological calcification mechanisms in a unified conceptual framework. In the current study, we attempt to lay some groundwork for such a holistic view. We argue that measurements of physiological processes such as foraminiferal calcification rates, the role of pumps and channels, and symbiont activity are needed to enhance our understanding of trace element partitioning. In addition, more experiments that target the effects of changing the major element composition of seawater are needed. For geological applications, it will also be important to consider the long-term or evolutionary response of foraminifera to environmental changes, which may differ from the short-term (single lifespan) responses we have described here.

5 Conclusions

Culture experiments with *O. universa*, *G. ruber* (pink), *G. sacculifer* and *G. bulloides* were conducted to quantify relationships between the trace element composition of planktic foraminiferal calcite and environmental growth conditions.

1. In *G. sacculifer*, the response of Sr/Ca to rising $[\text{CO}_3^{2-}]$ is ~12x greater in DIC experiments (constant pH) than in pH experiments (constant DIC). Predicted Sr/Ca changes across Pleistocene glacial cycles are smaller than analytical uncertainty; however, the Sr/Ca-DIC relationship may be useful for evaluating more dramatic carbon cycle changes earlier in the Cenozoic.

2. In *G. ruber*, Na/Ca increases ~1% per salinity unit, but no significant relationship is observed in *G. sacculifer*. According to the *G. ruber* calibration, an increase of 1 salinity unit should result in a Na/Ca increase of ~0.1 mmol/mol. This salinity increase, roughly the mean change predicted for the last glacial maximum, is within the estimated analytical uncertainty for Na/Ca (1.7% of 6 mmol/mol equals 0.1 mmol/mol Na/Ca). In addition, the Na/Ca relationships with S, T, and pH observed in culture all predict a Na enrichment during glacial periods. Therefore, independent proxies are needed to disentangle these separate influences on Na/Ca.

3. Incorporation of some of the smaller cations (Cd, Mn) decreases in *O. universa* as $[\text{CO}_3^{2-}]$ increases. Mg/Ca decreases with $[\text{CO}_3^{2-}]$ in pH experiments, consistent with previous work (Evans et al., 2016; Lea et al., 1999; Russell et al., 2004), but Mg/Ca increases slightly with $[\text{CO}_3^{2-}]$ in DIC experiments. This suggests that the response of Mg/Ca to the carbonate system does not solely depend upon $[\text{CO}_3^{2-}]$. Any carbonate system effects on Mg/Ca are expected to be within analytical error for most late Pleistocene records. In light of the small contribution and uncertain nature of carbonate system influences on paleo Mg/Ca records, corrections are not recommended.

4. Net calcification rates vary by species (*O. universa* > *G. sacculifer* > *G. ruber* > *G. bulloides*), ranging from 0.3 ± 0.1 to 6 ± 1 $\mu\text{g/day}$. Calcification rates do not respond consistently to any experimental parameter (temperature, salinity, pH, DIC, $[\text{CO}_3^{2-}]$). Calcification rate behavior in the species studied here contrasts with results of inorganic calcite precipitation experiments, indicating that foraminifera exert strong control over the rate of shell growth. On average, cultured foraminifera grow more slowly ($\sim 100\times$) than inorganic calcite in recent experiments.

5. In general, thermodynamic calculations fail to predict observed trace element behavior in cultured planktic foraminifera. Instead, our results suggest that a combination of thermodynamic and kinetic controls largely explain the behavior of some trace metals (Mn, Cd, Sr, Ba) and biological controls likely play a role in others (Mg, Cd). Our results highlight the need to further examine both kinetic and biologic controls on trace element proxies. In addition, it is essential to test these culture calibrations in the open ocean (e.g., through core-top and sediment trap studies) to confirm that the trace element behavior observed in the laboratory holds in the natural environment.

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FIGURES 1-14 (and S1, S2) in separate file.

TABLES in separate Excel file.

FIG. CAPTIONS:

Fig. 1. Summary of trace metal data from this study (circles) with results from previous studies.

Fig. 2. In four species, Sr/Ca responses to temperature and salinity are not significant ($p > 0.05$). When $[\text{CO}_3^{2-}]$ is increased by raising DIC and holding pH constant, the response of Sr/Ca to $[\text{CO}_3^{2-}]$ is $\sim 12\times$ greater than when $[\text{CO}_3^{2-}]$ is raised by increasing pH. This

suggests that Sr/Ca is not merely a simple function of $[\text{CO}_3^{2-}]$. Sr/Ca values are expected to differ between *O. universa* experiments due to differences in Sr/Ca of seawater between “modern” and “Paleocene” experimental seawater (black circles and diamonds, respectively).

Fig. 3. Sr/Ca of planktic foraminiferal calcite increases linearly with seawater Sr/Ca. Black circles represent data from culture experiments (this study, ambient DIC experiments, average $D_{\text{Sr}} = 0.16$); colored lines were drawn based on average D_{Sr} values determined in previous studies.

Fig. 4. Mg/Ca, Mn/Ca, and Cd/Ca generally decrease with increasing seawater $[\text{CO}_3^{2-}]$, with the effect decreasing or disappearing above ambient pH. Mg/Ca of *G. sacculifer* does not appear to respond to $[\text{CO}_3^{2-}]$. Trace element incorporation varies among species, with *O. universa* having the highest Mg/Ca and *G. bulloides* having the highest Mn/Ca and Cd/Ca.

Fig. 5. In this study, *O. universa* U/Ca decreases 0.51 ± 0.32 nmol/mol per $^{\circ}\text{C}$ ($R^2 = 0.9$), unlike previous work in which a temperature influence was not observed (Russell et al., 2004). U/Ca may also decrease with salinity, though this relationship is not as strong (0.51 ± 0.54 nmol/mol per salinity unit, $R^2 = 0.5$). Our carbonate ion relationship agrees well with trends observed for *O. universa* and *G. bulloides* in Russell et al. (2004).

Fig. 6. Li/Ca does not exhibit consistent or significant relationships with growth temperature, salinity, or carbonate ion across the experimental ranges in this study. Consistent Li/Ca offsets between species imply some form of biological control or influence on Li incorporation. Error bars represent $\pm 5.8\%$ RSD.

Fig. 7. In *G. ruber*, Na/Ca increases with salinity ($R = 0.99$, $p = 0.01$), however, the possible influence of other variables (temperature and carbonate ion) suggests that Na/Ca as a salinity proxy in *G. ruber* requires further testing. The lowest and highest CO_3^{2-} experiments correspond to pH values of ~ 7.8 and 8.7 (total scale), respectively. See Table S1 for full experimental details.

Fig. 8. Net average calcification rates ($\pm 1\sigma$) calculated from shell weights and number of growth days in culture ($\mu\text{g/day}$). Calcification rates vary among species: *G. bulloides* < *G. ruber* < *G. sacculifer* < *O. universa* (Table S1).

Fig. 9. Relationships between net calcification rates ($\pm 1\sigma$) and experimental parameters (temperature, salinity, $[\text{CO}_3^{2-}]$) are species specific. See text for full discussion.

Fig. 10. In inorganic calcites, Sr/Ca increases with calcification rate, and calcification rate increases as the ratio of CO_3^{2-} to Ca^{2+} in solution approaches 1 (Nehrke et al, 2007). When cultured *G. sacculifer* and *O. universa* data are plotted with the inorganic data, it appears that the DIC culture experiments (red circles) parallel the inorganic data, while the pH culture experiments follow a different trend (blue circles). Assuming that foraminifera vacuolize seawater and then raise its pH to 9 (total scale), $[\text{CO}_3^{2-}]$ increases

in the vacuoles (black arrows) and foraminiferal D_{Sr} from both pH and DIC experiments exhibit a correlation with CO_3^{2-} / Ca^{2+} similar to inorganic observations.

Fig. 11. Empirical partition coefficients ($D = [TE/Ca]_{calcite} / [TE/Ca]_{seawater}$) vs. net calcification rates. Black = *O. universa*; Green = *G. sacculifer*; Red = *G. ruber*; Blue = *G. bulloides*.

Fig. 12. In *G. sacculifer*, Mg/Ca increases with DIC (and also $[CO_2]$, $[CO_3^{2-}]$ and $[HCO_3^{2-}]$) in constant-pH, variable-DIC experiments. In these experiments, Mg/Ca increased with $[CO_3^{2-}]$, in contrast to the decrease with $[CO_3^{2-}]$ observed in pH experiments (c.f. Fig. 3). Thus the relationship between Mg/Ca and $[CO_3^{2-}]$ is not straightforward.

Fig. 13. Comparison of empirical partition coefficients for cultured foraminifera (this study, D_{foram} , circles) and inorganic calcites (previous studies, D_{inorg} , triangles). Averages are provided in Table S3. For a full list of sources for inorganic data, see Table S4. Effective ionic radii (Å) are from Shannon et al. (1969), LS = low spin radius, HS = high spin radius. Fig. after Onuma (1979) and Rimstidt (1998). Dashed-line boxes around Li^+ and Na^+ emphasize that these monovalent ions may not compete with Ca^{2+} for inclusion in the lattice; see text for full discussion.

Fig. 14. (A) In pH and DIC experiments with *G. sacculifer*, D_B is not controlled by net calcification rate. (B) All D_B for all experiments for which net calcification rates were calculated. (C) Same data as in B, except plotted as the partition coefficient suggested by Uchikawa et al. (2015). Boron partitioning into calcite is variable and, if anything, appears to decrease with increasing calcification rate in contrast to experiments with inorganic calcite. This suggests foraminifers actively control calcification rate and/or B incorporation.

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Figure 1

Figure 2

Figure 3

Calcite Sr/Ca (mmol/mol)

D_{Sr} in planktonic foraminiferal calcite

1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0

0

2

4

6

8

10

Seawater Sr/Ca (mmol/mol)

Average D values

— this study

— Delaney et al., 1985

— Lea et al., 1999

— Russell et al., 2004

— Dueñas-Bohórquez et al., 2009

● Cultured foraminifera (this study)

0.0

0.2

0.4

0.6

0.8

1.0

1.2

1.4

Figure 4

Figure 5

Figure 6

Figure 7

Figure 8

Figure 9

Figure 10

Inorganic calcite

—□— $D_{\text{Sr}}, \Omega = 5$

—■— $D_{\text{Sr}}, \Omega = 12$

Foraminiferal calcite

Measured values:

● $D_{\text{Sr}}, G. \text{ sacc}, \text{ changing DIC}$

● $D_{\text{Sr}}, G. \text{ sacc}, \text{ changing pH}$

◆ $D_{\text{Sr}}, O. \text{ universa}, \text{ Paleocene sw}$

—▶— Calculated internal solution

◆ Calculated internal solution

Figure 11

Figure 12

Figure 13

Figure 14