Environmental controls on B/Ca in calcite tests of the tropical planktic foraminifer species *Globigerinoides ruber* and *Globigerinoides sacculifer*

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ABSTRACT

The ratio of boron to calcium (B/Ca) in the calcite tests of planktic foraminifers may serve as a proxy for past seawater chemistry, but controls on B incorporation are not yet certain. Here we present the results of laboratory culture experiments with live specimens of *Globigerinoides ruber* (pink) and *Globigerinoides sacculifer*, which provide new insight into B incorporation controls. We find that in *G. sacculifer*, B/Ca increases with increasing pH (lower $[\text{HCO}_3^-]$, higher $[\text{CO}_3^{2-}]$ and $[\text{B}(\text{OH})_4^-]$), but decreases with increasing total dissolved inorganic carbon (DIC) (higher $[\text{HCO}_3^-]$ and $[\text{CO}_3^{2-}]$, constant $[\text{B}(\text{OH})_4^-]$). This suggests competition between aqueous boron and carbon species for inclusion into the calcite lattice. Similar to previous experiments with the subtropical-temperate *Orbulina universa*, B/Ca increases with salinity, but not with temperature. We evaluate possible carbonate system control parameters, and compare our tropical culture calibrations with new and published core-top data.

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1. Introduction

The ratio of boron to calcium (B/Ca) in fossil calcite tests of planktic foraminifers has recently been used to investigate the carbonate chemistry of ancient oceans (Foster, 2008; Palmer et al., 2010; Seki et al., 2010; Tripathi et al., 2009; Yu et al., 2007). The theoretical basis for this proxy stems from the pH-dependent concentration of dissolved borate ($\text{B}(\text{OH})_4^-$) and its subsequent incorporation into foraminiferal calcite (Hemming and Hanson, 1992). Controls on boron incorporation in several planktic species have been investigated through core-top studies (Foster, 2008; Ni et al., 2007; Yu et al., 2007), sediment-trap samples (Hendry et al., 2009), and culture experiments (Allen et al., 2011; Sanyal et al., 2001). Results vary by region and by species, and no consensus has yet been reached on the influence of temperature or individual carbonate system parameters (Foster 2008, Yu et al. 2007).

B/Ca has been applied as a proxy for either the concentration of carbonate ion ($[\text{CO}_3^{2-}]$) in seawater (Foster, 2008), or the ratio of aqueous borate to bicarbonate ($[\text{B}(\text{OH})_4^-]/[\text{HCO}_3^-]$), which can be used to estimate seawater pH if total dissolved boron and carbon concentrations are also known (Palmer et al., 2010; Tripathi et al., 2009; Yu et al., 2007). Combination of $[\text{CO}_3^{2-}]$ or pH with another

parameter (such as alkalinity or total dissolved inorganic carbon) then allows the entire carbonate system to be constrained. However, because carbonate system parameters often covary in modern seawater, core-top and culture experiment studies to date have not been able to tease apart their separate influences.

Yu et al. (2007) suggested that B incorporation increases with temperature in *Globorotalia inflata* and *Globigerina bulloides* derived from core-top sediments. By contrast, in *Globigerinoides ruber* and *Globigerinoides sacculifer*, B/Ca appears to decrease with temperature (Foster, 2008). In cultured *Orbulina universa*, B/Ca does not change significantly across an 8 °C temperature range (95% confidence level, Allen et al., 2011). Culture experiments also reveal that B/Ca in *O. universa* increases with salinity (Allen et al., 2011), but evaluation of a salinity effect on existing core-top specimens is difficult due to the correlation of salinity with other variables in the modern surface ocean. In summary, it is not clear whether planktic foraminiferal B/Ca is controlled by temperature, salinity, $[\text{CO}_3^{2-}]$, $[\text{B}(\text{OH})_4^-]/[\text{HCO}_3^-]$, or some combination of these or other parameters. To improve confidence in the interpretation of B/Ca, we need to understand which carbonate system parameter(s) controls B incorporation, and to quantify the influence of other environmental variables such as temperature and salinity.

Some basic principles provide a starting place when considering the process of trace element incorporation. Typically, relative rates of ion adsorption and desorption during crystal growth determine the amount of a chemical species (e.g., $\text{B}(\text{OH})_4^-$) that is

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ultimately incorporated into a solid (e.g., calcite). For inorganic calcites, adsorption scales with ion flux to the surface and the availability of kink sites, while detachment depends on the strength of an ion's bond to its neighbors, which is a function of temperature (De Yoreo and Vekilov, 2003). This suggests that dissolved ion concentrations and temperature both have the potential to influence B incorporation. However, to interpret geochemical signatures from the fossil record, we need to understand the trace element composition of calcite that has been secreted by a living organism. This requires that we consider an additional set of controls, because marine organisms exert considerable energy to nucleate and build carbonate tests from seawater (for a review, see Weiner and Dove, 2003). Several strategies are used by different calcifying species to create conditions favorable for mineral growth, which include: raising the saturation state (e.g., by increasing ionic strength or pH), regulating the concentrations of inhibitors or impurities, forming a preliminary amorphous carbonate phase, or providing organic templates for carbonate formation (Erez, 2003; Feng, 2011; Weiner and Addadi, 1997, 2011). These processes, especially those that influence the seawater carbonate system of a foraminifer's microenvironment, might influence B incorporation.

Here, we present the results of culture experiments with live specimens of *G. ruber* and *G. sacculifer*. We discuss both biological and inorganic processes that may control B incorporation into foraminiferal calcite. Because foraminiferal calcite does not typically form in equilibrium with seawater with respect to trace elements (Elderfield et al., 1996), we test and discuss empirical relationships rather than equilibrium partition coefficients. Finally, we compare B/Ca results from culture experiments with new sediment core-tops from the Gulf of Mexico and with published core-top results from the global ocean.

2. Methods

2.1. Culture experiments

Culture experiments were performed at the University of Puerto Rico's Marine Sciences Center on Isla Maguey during March and April 2010. Scuba divers hand-collected juvenile foraminifers 8 nautical miles offshore (17°52'N, 66°58'W) between 2 and 6 m water depths. During the collection period, average surface water temperature was 27.8 °C (range 27.5–28.5 °C), and salinity was 35.4 (range 35.0–35.8) on the practical scale (Lewis and Perkin, 1978; UNESCO, 1981). Immediately after collection, foraminifers were brought to the laboratory and identified. Specimens were measured under a light microscope (maximum diameter) and then transferred to experimental seawater. If no food was observed in foraminifers' spines, they were fed a one-day-old brine shrimp (*Artemia salina*). Subsequently, *G. ruber* (pink) and *G. sacculifer* juveniles were each fed a live *Artemia* nauplius every 2 days.

Most seawater used in these experiments was collected offshore and filtered (0.8 µm) to remove large particles. The one exception was our low-DIC experiment, which consisted of half natural and half artificial seawater. Experiments were designed to vary either temperature, salinity, or carbonate system parameters. In each experiment, ~20 *G. sacculifer* and ~50 *G. ruber* specimens were individually cultured in 120 mL glass jars. All jars were placed in temperature-controlled water baths topped with cool-white fluorescent lamps whose output was measured biweekly with a light meter. A strict diurnal light cycle was maintained throughout all experiments: 12 h light, 12 h dark. Over the course of 1–3 weeks, foraminifers added new chambers to their calcite tests to accommodate cytoplasm growth. Specimens were

observed daily through a 10 × hand lens to determine spine and symbiont presence, chamber formation, and degree of chamber-fill by cytoplasm. After an individual underwent gametogenesis, the remaining empty calcite test was rinsed in de-ionized water, dried, and archived for later analysis. The maximum diameter after incubation was measured to determine growth.

We lowered the salinity of ambient seawater to 33 by adding de-ionized water, and raised salinity to 40 by partial evaporation under a heat lamp at ~60 °C. Temperatures of 24, 26, and 30 °C were established in constantly-circulating water baths whose conditions were monitored by HOBO TidbiT® temperature loggers every 5 min. To raise or lower seawater pH, we added NaOH or HCl, respectively. In these experiments, raising or lowering pH also raised or lowered the dissolved $[\text{CO}_3^{2-}]$. To test the effect of varying carbonate ion separately from pH, we also conducted experiments that maintained constant pH but changed $[\text{CO}_3^{2-}]$ by varying the concentration of DIC. To raise DIC, we dissolved 0.75 g NaHCO_3 in 4 L ambient seawater, and then immediately added NaOH to return the solution to ambient pH of 8.0 (total scale). To lower DIC, we mixed two liters of ambient seawater ($\text{DIC}=2033 \mu\text{mol kg}^{-1}$) with two liters of synthetic seawater ($\text{DIC}=0 \mu\text{mol kg}^{-1}$) and titrated pH back to ambient seawater conditions (for 4L synthetic seawater recipe, see Supplementary material). Final DIC was not estimated assuming linear mixing of these two solutions, but instead was calculated from pH and alkalinity measurements made on the final natural-synthetic seawater mixture (details below).

To minimize gas-exchange with the atmosphere, jars were filled without a gas headspace, and then sealed with Parafilm® and tight-fitting (snap-cap) plastic lids. To monitor potential atmospheric CO_2 exchange during feeding (when caps must be removed), alkalinity and pH were measured at the beginning (2–3 water samples) and end of each experiment (3–8 water samples). Alkalinity and pH were measured using a Metrohm 809 open cell auto-titrator and pH meter, calibrated against NIST buffers and Dickson-certified alkalinity standards.

Calculation of the seawater carbonate system was performed with the MATLAB® program csys3.m (Zeebe and Wolf-Gladrow, 2001), modified by K. Allen to allow input of total dissolved boron (Uppström, 1974). Standard deviations (1σ) for experimental temperature were calculated from continuous TidbiT meter data measured every 5 min throughout each experiment ($n \approx 10,000$). Standard deviations for alkalinity and pH were calculated from suites of measurements made on experimental seawater throughout each experiment (average $n=13$). The 1σ measurement error given by Orion Star Thermo Scientific for the conductivity meter was 0.1. Confidence bounds ($\pm 1\sigma$) for each parameter (temperature, salinity, pH and alkalinity) were propagated through the full MATLAB calculation, yielding upper and lower estimates for all calculated carbonate system parameters (e.g., $[\text{CO}_2]$, $[\text{CO}_3^{2-}]$). Finally, these upper-lower estimate ranges were combined to yield a composite uncertainty value according to the equation below:

$$\sigma_A = \sqrt{([A]_U - [A]_L)_{\text{Temp}}^2 + ([A]_U - [A]_L)_{\text{sal}}^2 + ([A]_U - [A]_L)_{\text{pH}}^2 + ([A]_U - [A]_L)_{\text{Alk}}^2} \quad (1)$$

where A is the calculated parameter (e.g., $[\text{CO}_3^{2-}]$), and subscripts U and L indicate upper and lower estimates, respectively.

2.2. Gulf of Mexico samples

Sediment box cores were collected in 2007 from the Garrison (PE07-2) and Fisk Basins (PE07-5, PE07-6), and in 2003 from the Pigmy Basin (PBBC-1) by the R/V Longhorn (Richey et al., 2011). A radiocarbon-based age model was established by Richey et al. (2007), indicating a core-top age of ~1950 A.D. *Orbulina universa* and *G. sacculifer* specimens between 500 and 710 µm diameters

were picked from surface sediment samples. Surface seawater properties for these core sites were interpolated from modern transects published by the Gulf of Mexico and East Coast Carbon Cruise (Peng and Langdon, 2007). Alkalinity and DIC measurements were used to estimate the rest of the carbonate system using a modified version of csys3.m (same as above). Surface ocean DIC values could not be corrected for industrial CO_2 invasion because CFC data were not available for these sites.

2.3. Sample preparation

The final calcite tests produced by foraminifers in our culture experiments were composed of chambers grown in the open ocean before collection (under unconstrained conditions), and chambers added after insertion into experimental seawater (known conditions). To analyze only the calcite that grew in experiments, we used a medical scalpel blade and a light microscope to amputate the calcite chambers that were grown after collection under controlled conditions. Amputated chambers from each experimental condition were pooled to make a single sample. On average, each chamber weighs $20\text{ }\mu\text{g}$ for *G. sacculifer* and $5\text{ }\mu\text{g}$ for *G. ruber*, requiring approximately 20–80 chambers per experiment, respectively, to yield a total of $400\text{ }\mu\text{g}$ of calcite.

Cultured samples were cleaned according to the methods of Russell et al. (2004), using two 30-min treatments of a hot, buffered hydrogen peroxide solution (equal parts $0.1\text{ N NaOH} + 30\% \text{ H}_2\text{O}_2$, $70\text{--}80\text{ }^\circ\text{C}$) to remove organic matter.

Core-top samples were crushed, then rinsed and sonicated with Milli-Q⁺ water $5\times$ (or until no fine particles were visible) to remove clays. Samples were treated with a reductive solution (anhydrous hydrazine, ammonium hydroxide, and citric acid) to remove oxides, and an oxidative solution (hydrogen peroxide and sodium hydroxide) to remove organic matter following the modified protocol of Boyle and Keigwin (1985/1986) as outlined in Rosenthal et al. (1997).

Finally, all cleaned samples were rinsed 3 times with ultra-pure, B-filtered Milli-Q⁺ water, leached with 0.001 N HNO_3 , and rinsed again 3 times with Milli-Q⁺. Immediately prior to analysis, samples were dissolved in 0.65 N HNO_3 (trace element grade).

2.4. Analysis

Elemental ratios of calcite samples were determined on a sector-field inductively coupled plasma mass spectrometer (Thermo

Scientific Element XR) at Rutgers University. Precise and accurate determinations of B/Ca ratios may be compromised by both high B blanks and a memory effect. To reduce blank levels during the analysis, we used a cyclonic quartz spray chamber coupled with a sapphire injector, and we minimized memory effects by injecting ammonia gas directly into the spray chamber at a flow rate of 0.07 ml min^{-1} (Al-Amman et al., 2000). All reagents were made with B-free water. Typical B blank was $\sim 0.02\text{ ppb}$ or less than 3% of the foraminiferal content throughout the run. Standard solutions with differing $[\text{Ca}^{2+}]$ ($1.5\text{--}8\text{ mM}$) were run to quantify and correct for any matrix effects on trace element ratios. The influence of $[\text{Ca}^{2+}]$ on B/Ca was minor. Within-run precision of the matrix-corrected B/Ca data was 2.7%, 1.2%, and 3.0% as determined by duplicate analysis of three consistency standards at the beginning and end of the run.

3. Results

3.1. Culture experiments

3.1.1. Carbonate system

Between the low pH (7.49 ± 0.05) and high pH (8.45 ± 0.03) experiments, B/Ca increased from 134 to $212\text{ }\mu\text{mol mol}^{-1}$ in *G. ruber*, and from 87 to $137\text{ }\mu\text{mol mol}^{-1}$ in *G. sacculifer* (Fig. 1a). In these experiments, adding NaOH or HCl shifted not only pH but also the aqueous speciation of boron and carbon: $[\text{CO}_3^{2-}]$ increased from 66 to $498\text{ }\mu\text{mol kg}^{-1}$ (Fig. 1b); $[\text{B(OH)}_4^-]$ increased from 31 to $177\text{ }\mu\text{mol kg}^{-1}$ (Fig. 1c); $[\text{HCO}_3^-]$ decreased from 1896 to $1564\text{ }\mu\text{mol kg}^{-1}$, and alkalinity increased from 2061 to $2755\text{ }\mu\text{mol kg}^{-1}$ (Table 1).

The high-pH *G. sacculifer* experiment yielded enough calcite that we were able to combine several final sac chambers to make a separate sample. This final-chamber calcite had lower B/Ca ($94 \pm 5\text{ }\mu\text{mol mol}^{-1}$) than non-sac chambers from the same experiment ($145 \pm 8\text{ }\mu\text{mol mol}^{-1}$) (Table 1). A weighted average of sac and earlier chambers (based on initial calcite sample weights) yields a “with-sac” value of $133\text{ }\mu\text{mol mol}^{-1}$. This value is provided to facilitate direct comparison with other *G. sacculifer* samples whose sac chambers were not amputated.

Separate experiments that varied DIC while keeping pH constant at 8.0 (total scale) revealed a negative correlation between DIC and B/Ca, which decreased from 144 to $72\text{ }\mu\text{mol mol}^{-1}$ between 1046 and $4196\text{ }\mu\text{mol DIC kg}^{-1}$ seawater (Fig. 2).

Fig. 1. B/Ca of *G. ruber*, *G. sacculifer* and *O. universa* grown in experiments where pH was shifted by adding acid or base. In these experiments, $[\text{B(OH)}_4^-]$, $[\text{CO}_3^{2-}]$, and alkalinity all increase with pH. The B/Ca behavior of different species is similar, but offset.

Table 1

Seawater properties and B/Ca results for culture experiments. Each experimental sample comprises approximately 40–50 *G. ruber* (pink) and 20–30 *G. sacculifer* specimens. Elemental analyses were performed on samples composed only of calcite grown under experimental conditions. See text for details. Measured seawater parameters include temperature, salinity, pH, and alkalinity, which were used to calculate $[\text{CO}_3^{2-}]$, $[\text{B}(\text{OH})_4^-]$, DIC, and Ω_c . Uncertainties reported for measured seawater parameters represent 1-sigma standard deviations of all seawater measurements made throughout each experiment; uncertainties for calculated values represent the root-mean-square combined uncertainty from measured parameters. B/Ca_R and B/Ca_{CB} indicate values on the Rutgers University and Cambridge-Bristol University scales, respectively (see Eq. (8)).

Species	Experiment	Sample	Temp (°C)	+/-	Salinity	HCO ₃ ⁻ (μmol/kg)	+/-	CO ₃ ²⁻ (μmol/kg)	+/-	B(OH) ₄ ⁻ (μmol/kg)	+/-	DIC (μmol/kg)	+/-	ALK (μmol/kg)	+/-	Ω _c	+/-	pH Total	+/-	B/Ca _R (μmol/mol)	+/-	B/Ca _{CB}
<i>G. sacculifer</i>	Ambient	1a	25.7	0.4	35.4	1800	61	221	22	92	9	2033	47	2342	18	5.3	0.5	8.04	0.06	95.9	5.3	89.4
		1b	25.7	0.4	35.4	1800	61	221	22	92	9	2033	47	2342	18	5.3	0.5	8.04	0.06	90.0	4.9	83.9
	Low temperature	2	24.0	0.2	35.6	1823	36	212	11	90	5	2047	31	2344	14	5.1	0.3	8.04	0.03	91.9	5.1	85.7
	High temperature	4	29.3	0.2	35.2	1809	83	220	34	89	14	2042	53	2347	4	5.4	0.8	7.98	0.04	92.9	5.1	86.6
	Low salinity	5	25.7	0.2	33.0	1730	195	184	77	78	33	1927	131	2183	26	4.5	1.9	7.99	0.12	84.3	4.6	78.7
	High salinity	7	25.5	0.3	40.0	1963	77	265	19	108	7	2240	78	2609	41	6.1	0.4	8.04	0.02	119.0	6.5	110.7
	Low DIC	8	25.7	0.2	35.4	947	104	91	32	76	27	1046	84	1210	29	2.2	0.8	7.93	0.10	144.0	7.9	133.7
	High DIC	9	25.6	0.3	35.4	3694	158	480	36	96	6	4196	166	4757	89	11.6	0.9	8.07	0.02	72.3	4.0	67.6
	Low pH	12a	25.7	0.5	35.5	1896	44	66	14	31	6	2005	43	2061	15	1.6	0.3	7.49	0.05	86.2	4.7	80.4
		12b	25.7	0.5	35.5	1896	44	66	14	31	6	2005	43	2061	15	1.6	0.3	7.49	0.05	88.4	4.9	82.5
	High pH	13a	25.7	0.5	35.4	1564	95	498	38	177	14	2065	64	2755	21	12.0	0.9	8.45	0.03	145.0	8.0	134.7
		13b	25.7	0.5	35.4	1564	95	498	38	177	14	2065	64	2755	21	12.0	0.9	8.45	0.03	129.5	7.1	120.4
	Final chamber	13c	25.7	0.5	35.4	1564	95	498	38	177	14	2065	64	2755	21	12.0	0.9	8.45	0.03	94.2	5.2	87.8
<i>G. ruber</i> (pink)	Ambient	1	25.7	0.4	35.4	1800	61	221	22	92	9	2033	47	2342	36	5.3	0.5	8.04	0.01	141.9	7.8	131.8
	Low temperature	2	24.0	0.2	35.6	1823	36	212	11	90	5	2047	31	2344	28	5.1	0.3	8.04	0.03	127.5	7.0	118.6
	High temperature	4	29.3	0.2	35.2	1809	83	220	34	89	14	2042	53	2347	9	5.4	0.8	7.98	0.04	139.2	7.7	129.3
	Low salinity	5	25.7	0.2	33.0	1730	195	184	77	78	33	1927	131	2183	26	4.5	1.9	7.99	0.12	121.4	6.7	112.9
	High salinity	7	25.5	0.3	40.0	1963	77	265	19	108	7	2240	78	2609	41	6.1	0.4	8.04	0.02	154.8	8.5	143.6
	Low pH	12	25.7	0.5	35.5	1896	44	66	14	31	6	2005	43	2061	15	1.6	0.3	7.49	0.05	134.2	7.4	124.7
	High pH	13	25.7	0.5	35.4	1564	95	498	38	177	14	2065	64	2755	21	12.0	0.9	8.45	0.03	212.4	11.7	196.7

Fig. 2. When DIC is raised by addition of NaHCO_3 and seawater pH is held constant, $[\text{B}(\text{OH})_4^-]$ remains constant and $[\text{CO}_3^{2-}]$ increases. In these experiments, B/Ca of *G. sacculifer* decreases (black triangles). By contrast, when seawater pH is raised and DIC is held constant, both $[\text{B}(\text{OH})_4^-]$ and $[\text{CO}_3^{2-}]$ are increased, and B/Ca increases (blue triangles). These results suggest that $\text{B}(\text{OH})_4^-$ and CO_3^{2-} ions may compete for the same site in the calcite lattice. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.1.2. Temperature and salinity

B/Ca does not exhibit a significant relationship with temperature in *G. ruber* ($R^2 = 0.38$, $p = 0.57$) or *G. sacculifer* ($R^2 = 0.02$, $p = 0.87$) (Fig. 3). In both species, B/Ca ($\mu\text{mol mol}^{-1}$) increases with salinity:

G. ruber (pink)

$$\text{B/Ca} = 4.51 \times S - 23.6, \quad R^2 = 0.91, \quad p = 0.20 \quad (2)$$

G. sacculifer

$$\text{B/Ca} = 5.10 \times S - 85.9, \quad R^2 = 0.96, \quad p = 0.02 \quad (3)$$

Standard deviations of water bath temperatures, measured every 5 min for the duration of each experiment (lasting 3–5 weeks) were less than $\pm 0.5^\circ\text{C}$. Between the beginning and end of each experiment, pH drifted no more than 0.05 units. Exceptions are the low-salinity experiment, in which pH decreased by 0.15 units and the low-pH experiment, in which pH increased by 0.07 units. These changes were likely caused by CO_2 exchange during brief intervals when lids were opened for feeding, and may be strongest in these experiments because they were farthest from equilibrium with atmospheric pCO_2 . For display and interpretation of all results, we used the average of initial and final experimental conditions.

3.2. Gulf of Mexico core-top samples

Estimated average surface seawater conditions (0–50 m below sea surface) for both core-top locations are 27.9°C , 35.9 salinity, $2040 \mu\text{mol kg}^{-1}$ DIC and $2386 \mu\text{mol kg}^{-1}$ alkalinity. *G. sacculifer* and *G. universa* B/Ca values fall between 111 and $122 \mu\text{mol mol}^{-1}$ and 71 and $76 \mu\text{mol mol}^{-1}$, respectively (Table 2).

4. Discussion

4.1. Carbonate system

Adding acid (base) to experimental seawater resulted in lower (higher) pH, $[\text{B}(\text{OH})_4^-]$ and $[\text{CO}_3^{2-}]$ of the solution, and lower

Fig. 3. B/Ca increases with salinity in all three cultured species: *G. ruber* ($R^2=0.91$, $p=0.20$), *G. sacculifer* ($R^2=0.96$, $p=0.02$), and *O. universa* ($R^2=0.63$, $p=0.03$). B/Ca does not exhibit a strong relationship with temperature in *G. ruber* ($R^2=0.38$, $p=0.58$) or *G. sacculifer* ($R^2=0.02$, $p=0.87$), and increases slightly in *O. universa* ($R^2=0.62$, $p=0.11$).

Table 2

Results of core-top samples from the Gulf of Mexico. Seawater parameters were estimated from the 2007 GOMECC Report, assuming foraminiferal depth habitat of 0–50 m. All foraminifers were derived from the 500 to 710 μm size fraction.

Species	Core	Lat (N)	Long (W)	Temperature (°C)	Salinity	DIC (μmol/kg)	ALK (μmol/kg)	B/Ca _R (μmol/mol)	+/-	B/Ca _{CB} (μmol/mol)
<i>G. sacculifer</i>	PE07-6I	27.417	92.117	27.89	35.83	2040	2386	121.7	6.1	113.2
	PE07-5I	27.550	92.167	27.88	35.82	2040	2386	113.2	5.7	105.4
	PE07-6II	27.417	92.117	27.89	35.83	2040	2386	110.9	5.5	103.2
<i>O. universa</i>	NPBC	27.194	91.409	27.96	35.99	2038	2387	75.7	3.8	70.7
	PE07-6I	27.417	92.117	27.89	35.83	2040	2386	72.5	3.6	67.8
	PE07-6II	27.417	92.117	27.89	35.83	2040	2386	70.8	3.5	66.2

(higher) B/Ca of both *G. sacculifer* and *G. ruber* calcite (Fig. 1). These observations are consistent with B/Ca incorporation behavior of *O. universa* (Allen et al., 2011), and suggest a common control across these species. Higher seawater $[B(OH)_4^-]$ could have led to greater B inclusion, and/or higher $[CO_3^{2-}]$ could have caused more rapid calcification rates, which might also lead to greater incorporation of B. To investigate these possibilities, we performed another set of experiments with *G. sacculifer*, in which we raised $[CO_3^{2-}]$ by increasing DIC while holding pH and $[B(OH)_4^-]$ constant. These experiments reveal that, all else being equal, B/Ca decreases as $[CO_3^{2-}]$ increases (Fig. 2).

These results imply that calcite saturation state ($\Omega_c = ([Ca^{2+}][CO_3^{2-}])_{seawater} / ([Ca^{2+}][CO_3^{2-}])_{saturation}$) is not a major control on B/Ca. Between the low and high DIC experiments, Ω_c increased from 2.2 to 11.6. The saturation state increase between the low and high pH experiments was similar: from 1.6 to 12.0. Although we could not measure foraminiferal calcification rates directly, calcification rate is expected to increase with saturation (DePaolo, 2011; Zuddas and Mucci, 1998). If so, calcification in both the “high $[CO_3^{2-}]$ ” seawater ($\Omega=11.6$) and the “high pH” seawater ($\Omega=12.0$) should have been more rapid. However, the high DIC experiment yields very low B/Ca, and high pH yields very high B/Ca. Instead of a saturation state control, the B/Ca of calcite appears to be primarily influenced by the relative abundances of $B(OH)_4^-$ and carbonate species in seawater. In the following discussion, we test different combinations of $[B(OH)_4^-]$, $[HCO_3^-]$ and $[CO_3^{2-}]$ as control parameters. If one or some combination of these parameters consistently controls B/Ca, such a relationship may be useful for paleoclimate reconstructions.

First, we consider a scenario in which $B(OH)_4^-$ and CO_3^{2-} are competing with each other for inclusion in the calcite lattice.

If B/Ca increases with $[B(OH)_4^-]$ and decreases with $[CO_3^{2-}]$, then B/Ca should exhibit a positive relationship with $[B(OH)_4^-]/[CO_3^{2-}]$. However, B/Ca increases with $[B(OH)_4^-]/[CO_3^{2-}]$ in the pH experiments, and decreases with $[B(OH)_4^-]/[CO_3^{2-}]$ in the DIC experiments (Fig. 5G). This inconsistent behavior indicates $[B(OH)_4^-]/[CO_3^{2-}]$ is an incorrect or incomplete control parameter.

If we replace $[CO_3^{2-}]$ with $[HCO_3^-]$ (giving $[B(OH)_4^-]/[HCO_3^-]$), then the B/Ca relationships in the pH and DIC experiments become consistent (Fig. 5H). This unified B/Ca behavior suggests that HCO_3^- is the dominant carbon species in competition with $B(OH)_4^-$ for inclusion in the calcite lattice, which is consistent with the original incorporation stoichiometry hypothesized by Hemming and Hanson (1992).

In a third scenario, CO_3^{2-} and HCO_3^- both compete with $B(OH)_4^-$ during calcite growth. Near ambient seawater pH (~ 8.0 total scale), $[CO_2]$ is very small, so $DIC \approx [CO_3^{2-}] + [HCO_3^-]$. In Fig. 5I, B/Ca of both pH and DIC experiments is plotted against $[B(OH)_4^-]/DIC$. The $[B(OH)_4^-]/DIC$ and $[B(OH)_4^-]/[HCO_3^-]$ calibrations are very similar, which is not surprising because $[HCO_3^-]$ is the dominant component of DIC in seawater between pH 6 and 9. Given the available data, it is difficult to discern which calibration parameter ($[HCO_3^-]$ or DIC) most accurately quantifies the control on B incorporation. A key unknown is the amount of CO_3^{2-} incorporated relative to HCO_3^- . If CO_3^{2-} incorporation is negligible, then $[B(OH)_4^-]/[HCO_3^-]$ is the more appropriate control parameter. On the other hand, if a mixture of CO_3^{2-} and HCO_3^- is incorporated into calcite, then the reality may lie somewhere between $[B(OH)_4^-]/[HCO_3^-]$ and $[B(OH)_4^-]/DIC$.

For now, let us assume that both HCO_3^- and CO_3^{2-} are involved in calcification, and explore the possibility of $[B(OH)_4^-]/DIC$ control on B/Ca. In three species—*G. ruber* and *G. sacculifer* (this

study), and *O. universa* (Allen et al., 2011)—B/Ca increases with $[B(OH)_4^-]/DIC$. This relationship may be expressed as a simple linear regression of the form $B/Ca = m \times ([B(OH)_4^-]/DIC) + b$. This allows a slightly better fit than a multivariate regression of the form $B/Ca = m_1 \times [B(OH)_4^-] + m_2 \times DIC + b$. Here, we report linear fits to culture data that have been normalized to $S=35$:

G. ruber (pink)

$$B/Ca = [B(OH)_4^-]/DIC \times 1152_{-1437}^{3740} + 104_{-43}^{251}, \quad R^2 = 0.89 \quad (4)$$

G. sacculifer

$$B/Ca = [B(OH)_4^-]/DIC \times 853_{-461}^{1244} + 63_{-43}^{82}, \quad R^2 = 0.79 \quad (5)$$

O. universa

$$B/Ca = [B(OH)_4^-]/DIC \times 473_{-369}^{587} + 53_{-46}^{60}, \quad R^2 = 0.97 \quad (6)$$

where B/Ca is in $\mu\text{mol mol}^{-1}$ and $[B(OH)_4^-]/DIC$ is unitless. Salinity normalization was done for *G. ruber* and *G. sacculifer* using Eqs. (2) and (3), respectively, and for *O. universa* using the equation given in Fig. 3(a) from Allen et al. (2011). Upper and lower 90% confidence intervals are given next to fit parameters in super- and sub-script notation, respectively. Eq. (4) includes the high- and low-DIC experiments, and all *G. sacculifer* data include sac chambers (to be consistent with the other with-sac samples, the weighted-average of amputated sac-chambers and ontogenetic calcite B/Ca is used as the high-pH experiment value). These equations will be tested against core-top B/Ca data, and their down-core applicability will be discussed in Section 4.7.

4.2. Salinity

B/Ca increases with salinity at rates of 4.5 and 5.1 $\mu\text{mol mol}^{-1}$ per salinity unit in *G. ruber* and *G. sacculifer*, respectively. A positive relationship was also observed in *O. universa* (2.6 $\mu\text{mol mol}^{-1}$ per salinity unit, Allen et al., 2011). This raises the question: if all dissolved elements are concentrated equally as salinity is raised, why does B incorporation increase? Below, we explore a few possibilities.

Salinity influences the equilibrium constants of dissolved boron and carbon species, described by K_B , K_1 , and K_2 (Dickson, 1990; Lueker et al., 2000; Mehrbach et al., 1973). In our culture experiments, salinity ranged from 33 to 40, corresponding to $[B(OH)_4^-]/DIC$ of 0.041 and 0.048, respectively. Given this increase in $[B(OH)_4^-]/DIC$ of ~ 0.007 , the calibrations from carbonate system experiments predict B/Ca increases of 9 and 7 $\mu\text{mol mol}^{-1}$ for *G. ruber* and *G. sacculifer*, respectively. However, the increases observed in salinity experiments are larger: 33 and 35 $\mu\text{mol mol}^{-1}$. This implies that the influence of salinity on equilibrium constants cannot fully explain our observations, and that there is an additional salinity effect at work.

The B content of mollusk shells also increases with salinity (Furst et al., 1976) by approximately 1.7 $\mu\text{mol mol}^{-1}$ B/Ca per salinity unit, though the mechanism for this effect is uncertain. Because the calcification strategies of mollusks and foraminifera differ (Feng, 2011), their common B increase may be most easily explained by a common, inorganic mechanism rather than a biogenic one. Indeed, Kitano et al. (1978) observed that the boron content of inorganically-precipitated calcite crystals increases with salinity. Because Kitano et al. (1978) raised salinity simply by modifying the concentration of NaCl (not dissolved B), the resulting increase in [B] of calcite points to some mechanism involving ionic strength and/or ion–ion or ion–surface interactions. It is possible that the mechanism for a salinity effect on the B content of inorganic calcite crystals is also responsible for the response seen in marine biominerals. Although it is difficult to compare the results of Kitano's inorganic experiments with our

cultures quantitatively, because the pH of their solutions was not held constant during calcite precipitation (ranging from 7.5 to 8.2, uncertain scale), their results do point towards an inorganic origin of the salinity effect on B/Ca.

A salinity effect on B incorporation might stem from the influence of seawater ionic strength on individual ion activities. The ionic strength of seawater scales with the total amount and charge of ions dissolved in solution. It influences the activity (i.e. effective concentration, or tendency to react) of different ions in different ways because each ion has unique properties that dictate its behavior in solution—its attraction to other ions, its hydration sphere, etc. If $B(OH)_4^-$, CO_3^{2-} , and HCO_3^- compete for inclusion into calcite's carbonate ion site, then changes in the relative activities of these three ions with salinity might explain the observed change in B incorporation. Using an ion pairing model, Millero and Schreiber (1982) estimated activity coefficients for $B(OH)_4^-$, HCO_3^- and CO_3^{2-} in seawater between salinities of 1–40. We can combine these coefficients with typical ion concentrations in seawater to estimate ion activities according to the equation

$$\alpha = \gamma_i \times m_i \quad (7)$$

where γ is the activity coefficient, m is the concentration, and α is the activity (effective concentration). This calculation yields different ion activities at higher salinity: CO_3^{2-} activity remains roughly constant, while $B(OH)_4^-$ and HCO_3^- activities both increase (Fig. 4). Higher ion activity leads to greater incidence of bonding with the growing calcite surface (De Yoreo and Vekilov, 2003), so higher borate activity should lead to higher B incorporation into the solid. However, the effective concentration increase in HCO_3^- is much larger than that of $B(OH)_4^-$ (Fig. 5). If HCO_3^- is the dominant species competing with $B(OH)_4^-$ for inclusion into the calcite lattice, then it seems unlikely that changes in ion activity can explain our observed increase in B/Ca with salinity.

Fig. 4. The ionic strength of seawater increases with salinity, and influences the activity (i.e. effective concentration, or tendency to react) of dissolved ions. In this figure, ambient $B(OH)_4^-$, CO_3^{2-} , and HCO_3^- concentrations (gray lines) have been combined with activity coefficients in seawater to estimate ion activities (black lines). The activity of all three ions increases at higher salinity, but the activity of HCO_3^- increases to a much greater degree than that of $B(OH)_4^-$ and CO_3^{2-} . These results cannot explain the observed salinity effect on B/Ca. See text for details.

Fig. 5. B/Ca of *O. universa*, *G. ruber*, and *G. sacculifer* plotted against three different dissolved ion ratios. *G. sacculifer* was the only species with which we performed increasing pH/constant DIC experiments (blue triangles) and constant pH/increasing DIC experiments (black triangles). B/Ca patterns in the two experiments differ when $[B(OH)_4^-]/[CO_3^{2-}]$ is plotted as the controlling parameter (A), but become consistent when $[B(OH)_4^-]/[HCO_3^-]$ is used (B). If DIC replaces $[HCO_3^-]$, the relationship is similar (C). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

On the other hand, the activity of carbonate ion increases less with salinity than that of borate ion. This may be consistent with a role of carbonate ion in foraminiferal calcification.

In sum, the B/Ca increase observed in salinity experiments appears too large to be explained by salinity's effect on equilibrium constants or ion activities. While we cannot fully explain its origin, we recommend that the influence of salinity on B/Ca be considered when interpreting sediment records.

4.3. Temperature

Temperature influences the equilibrium constants K_1 , K_2 , and K_B , which in turn affect the $[B(OH)_4^-]/[DIC]$ ratio of seawater. In prior culture experiments performed with *O. universa* on Catalina Island (Allen et al., 2011), calculated $[B(OH)_4^-]/[DIC]$ of culture seawater increased by 0.01 between 18 and 26 °C. This difference, according to our carbonate system calibrations, should translate into a B/Ca increase of 4 $\mu\text{mol mol}^{-1}$ for *O. universa*. Between 24 and 30 °C in Puerto Rico, $[B(OH)_4^-]/[DIC]$ increases by 0.008. This difference should translate into B/Ca increases of 9 and 7 $\mu\text{mol mol}^{-1}$ for *G. ruber* and *G. sacculifer*, respectively. These estimated differences are small and consistent with the insignificant temperature relationships of B/Ca in *G. ruber* ($R^2=0.38$, $p=0.57$), *G. sacculifer* ($R^2=0.02$, $p=0.87$) and *O. universa* ($R^2=0.62$, $p=0.11$). Thus, although B/Ca is expected to increase slightly due to temperature's influence on equilibrium constants, changes are too small to be resolved by current analytical techniques across the temperature ranges provided in our experiments. Our observations confirm the earlier inference of Foster (2008), who argued that temperature cannot be the primary control on

B/Ca because core-top and down-core samples from the Atlantic are characterized by markedly different B/Ca-temperature relationships.

4.4. Species differences

When grown at ambient seawater pH (~ 8.0 , total scale), *G. ruber*, *G. sacculifer*, and *O. universa* tests yield B/Ca values of 142, 93, and 62 $\mu\text{mol mol}^{-1}$, respectively (corresponding to 140, 91, and 66 $\mu\text{mol mol}^{-1}$ when normalized to $S=35$ using the calibrated salinity relationships). These large and consistent B/Ca differences between foraminiferal species grown in the same seawater composition indicate that these organisms actively influence B incorporation. A better understanding of the biological controls would improve our ability to predict whether primary seawater variables (like salinity) will be unaffected by the organism's biological controls and be preserved in fossil calcite.

The boron isotopic composition $\delta^{11}\text{B}$ of foraminiferal calcite increases with seawater pH, and individual species' calibrations are also offset from each other (Hönisch et al., 2007). At any given seawater pH value, cultured *O. universa* have both lower B/Ca and lower $\delta^{11}\text{B}$ than *G. sacculifer* (Sanyal et al., 2001, 1996). It is possible that these species offsets are caused by internal biological controls such as ion pumping, but such processes are currently not well understood in planktic foraminifera. It is interesting to note that across a wide seawater pH range (~ 7 – 9), $\delta^{11}\text{B}$ offsets between species are roughly constant, but B/Ca offsets are not.

It is also interesting that regressions of B/Ca against these environmental control parameters (Figs. 1, 3 and 4, Eqs. (2)–(6)) do not intersect the origin. This is not an artifact of using $[B(OH)_4^-]/$

DIC as the control parameter, as calibrations versus $[B(OH)_4^-]/[HCO_3^-]$ yield similar results. In a conceptual model where B in calcite comes exclusively from $[B(OH)_4^-]$ in solution, B/Ca should approach zero when $[B(OH)_4^-]$ approaches zero. It is possible that the relationships are not linear, or that the relationship between B/Ca and seawater composition is complicated by active biological inclusion or exclusion of ions. It is also possible that a small amount of boric acid ($B(OH)_3$) is incorporated at low pH (Allen et al., 2011; Klochko et al., 2009). More work is needed to rigorously assess this possibility, and to determine the best form of the curve fit.

4.5. Geochemical peculiarity of *G. sacculifer*'s sac-like chamber

Globigerinoides sacculifer often produces a final, “sac-like” chamber a day or two before gametogenesis (Bé, 1980; Caron et al., 1990). A few hours before releasing gametes, individuals (both with and without this chamber) drop their spines and secrete a final biomineral layer over the entire outer test surface. This layer, known as gametogenic calcite, is estimated to contribute up to ~30% of the total test weight (Bé, 1980; Caron et al., 1990). Final sac chambers amputated from individuals in our high-pH experiment yield a B/Ca value of $94 \mu\text{mol mol}^{-1}$, ~43 $\mu\text{mol mol}^{-1}$ lower than non-sac chambers from the same experiment. While it should be noted that we did not analyze final non-sac chambers, and therefore cannot determine whether this observation is exclusive to sac chambers or universal to all final chambers, potential explanations for B depletion in these chambers involve changes in symbiont activity and/or calcification mechanism prior to gametogenesis.

Symbiont photosynthesis removes CO_2 from a foraminifer's microenvironment, raising local pH and $[\text{CO}_3^{2-}]$. This enhances calcification (Bé et al., 1982), increases $\delta^{11}\text{B}$ (Hönisch et al., 2003), and decreases test $\delta^{18}\text{O}$ (Bemis et al., 1998). In *G. sacculifer*, the sac chamber has higher $\delta^{18}\text{O}$ than the rest of the test, and the enrichment is greater when the chamber is secreted closer in time to gamete release (Spero and Lea, 1993). If a foraminifer possesses fewer symbionts when the sac chamber is formed (or if it has sunk into deeper water with lower light levels), photosynthetic CO_2 sequestration would be lower, leading to lower $[\text{CO}_3^{2-}]$ and pH, and perhaps higher $\delta^{18}\text{O}$ and lower B/Ca of foraminiferal calcite. Changes in symbiont numbers through a foraminifer's life cycle are not well known, but in order to survive, a foraminifer's symbionts must abandon their host before it sinks too far from the photic zone. If abandonment begins before or during final-chamber formation, $[B(OH)_4^-]$ and $[B(OH)_4^-]/\text{DIC}$ within a foraminifer's microenvironment would likely decrease. If the pH change due to loss of symbionts were of the same magnitude as that observed between darkness and full light conditions (Jørgensen et al., 1986), $[B(OH)_4^-]$ would decrease by ~90 $\mu\text{mol mol}^{-1}$ and $[B(OH)_4^-]/\text{DIC}$ by ~0.04 mol mol^{-1} . According to Eq. (4), this would lead to a lowering of B/Ca by approximately 40 $\mu\text{mol mol}^{-1}$. This is similar to the difference observed between sac and ontogenetic chambers. To test this mechanism, microelectrodes could be used to monitor pH within the *G. sacculifer* microenvironment throughout the life cycle. Previously-applied techniques for counting symbionts require destruction of both symbionts and host (Spero and Parker, 1985), but if non-destructive methods were applied, symbiont counts throughout the lives of individuals could be accomplished to help resolve this issue.

Differences in the formation of gametogenic and ontogenetic calcite might also explain the lower B/Ca of sac-chamber calcite. After spines are discarded, the rhizopodial network collapses and gametogenic calcite forms (Bé, 1980). This calcite is lumpy and uneven (Bé, 1980; Bé and Hemleben, 1970), suggesting a different biomineralization process from the neatly-layered and structured

ontogenetic calcite. If gametogenic calcite is deposited evenly across the entire test, the final chamber would likely receive a greater proportion than the other chambers, because it is larger and thinner, and does not have as many ontogenetic layers as the older chambers (Bé and Hemleben, 1970; Erez, 2003). However, the B/Ca composition of *G. sacculifer*'s gametogenic calcite is uncertain. In *O. universa*, B/Ca tends to decrease towards the outermost part of the test, but not always (Allen et al., 2011), and the boundary between gametogenic and ontogenetic calcite in specimens analyzed to date is not clear.

4.6. Comparison with core-top data

To evaluate whether our culture calibrations are applicable to core-top specimens, we use Eqs. (4), (5) and (6) as well as oceanographic data to predict B/Ca of the core-top *G. ruber* and *G. sacculifer* studied by Foster (2008), Ni et al. (2007), Tripathi et al. (2009), and our samples from the Gulf of Mexico. To compare directly the culture and core-top B/Ca data, we must account for a systematic analytical B/Ca offset between data measured at Rutgers University, where most cultured samples were analyzed, and Bristol University, where most core-top data were measured. The conversion equation is derived from an inter-laboratory comparison between Rutgers and Cambridge (Yair Rosenthal and Jimin Yu, personal communication), including calcite from both *G. ruber* and *G. sacculifer*:

$$(B/Ca)_{\text{Rutgers}} = 1.085 \times (B/Ca)_{\text{Cambridge}} - 1.09 \quad (8)$$

We assume that this equation ($R^2=0.94$, $n=7$) is also applicable to data derived from Bristol, because no B/Ca offset has been observed between Bristol and Cambridge (Rae et al., 2011). On average, these adjustments raise Cambridge and Bristol *G. ruber*, *G. sacculifer*, and *O. universa* B/Ca values by ~7%.

To estimate the pre-industrial conditions in which foraminifers likely grew, Foster (2008) subtracted anthropogenic CO_2 reported by the Global Ocean Data Analysis Project (GLODAP; Key et al., 2004) from DIC, and Ni et al. (2007) assumed surface ocean CO_2 equilibrium with the pre-industrial atmosphere. Invasion of anthropogenic CO_2 in the Gulf of Mexico has not been quantified, but the modern age of the sediment at our core-top sites (Richey et al., 2007) suggests that adjustments are likely to be minor or unnecessary.

The resulting comparison between predicted and measured B/Ca values is illustrated in Fig. 6. All measured B/Ca values, both cultured and core-top, have been normalized to $S=35$ and, if necessary, converted to the Rutgers B/Ca scale using Eq. (8). After accounting for salinity differences, there is good agreement between predicted and measured B/Ca ($R^2=0.7$, $p<0.01$). Most measured core-top values for *O. universa* and *G. sacculifer* are similar to values predicted by culture calibrations (average offsets are 4 and 15 $\mu\text{mol mol}^{-1}$, respectively) but values predicted for core-top *G. ruber* deviate by up to 60 $\mu\text{mol mol}^{-1}$ from predicted values. The discrepancy observed for core-tops may suggest that our experiments still fall short of identifying all environmental controls on B/Ca and/or that we need to revisit the growth conditions assumed for planktic foraminifers, in particular the depth habitat of *G. ruber*.

We consider the following possible explanations for discrepancies between predicted and measured B/Ca of core-top samples. First, some of the core-top sediments may have accumulated as early as 4000 yr ago (Foster, 2008). Surface seawater parameters ascribed to core-top sites were measured during the 20th and 21st centuries (Key et al., 2004), and although the surface carbonate system values in these datasets have been corrected for industrial CO_2 invasion (Sabine et al., 2004), it is

Fig. 6. Culture calibrations are combined with surface seawater properties (GLODAP database, Key et al., 2004) to predict foraminiferal B/Ca values at several core-top locations using culture calibration Eqs. (4), (5), and (6) ($R^2=0.7$). After normalizing to $S=35$, most B/Ca predictions are similar to measured values (Foster, 2008; Ni et al., 2007; Tripathi et al., 2009), with *G. ruber* exhibiting the largest offsets. Differences between predicted and measured B/Ca may be due to uncertain foraminifer depth habitats, differences in age between seawater measurements and core-top sediment age, calibration limitations, or an unidentified environmental control on B/Ca. Gray bar with arrows represents the B/Ca range expected in *G. ruber* for a pH range of 8.0–8.15 (assuming $T=25^\circ\text{C}$, $S=35$, $\text{DIC}=1950\ \mu\text{mol kg}^{-1}$). All B/Ca shown here have been converted to the “Rutgers scale” according to Eq. (8) (see text for details).

unclear how well these pre-industrial oceanographic data approximate mid-Holocene conditions.

Second, even if surface seawater conditions at the time of core-top deposition are well-approximated by pre-industrial surface conditions, it is still difficult to determine the seawater environment in which core-top-derived planktic foraminifers grew. Foraminifers migrate vertically through the water column during their life cycle, which can expose them to different seawater conditions. At all core-top sites in Fig. 6, $[\text{B}(\text{OH})_4^-]/\text{DIC}$ decreases with depth (calculated using the GLODAP database). Thus, the offset between predicted and measured B/Ca for *G. ruber* might be explained if these specimens lived at greater depths than assumed. Applying our *G. ruber* calibration equation to depth profiles of $[\text{B}(\text{OH})_4^-]/\text{DIC}$ at each core-top site allows us to predict B/Ca values of foraminifers growing at different depths in the water column. Comparing the core-top B/Ca values measured by Foster (2008) with these estimated profiles yields habitat depths of 61, 48, 41, and 38 m for sites 925, 664, 668, and 999A, respectively. These depths differ from the widely adopted surface—10 m growth habitat for *G. ruber* (e.g., Foster, 2008) but are within the habitat ranges observed in plankton tow studies (Fairbanks et al., 1980; Williams et al., 1981), and may represent an average of depths at which the foraminifers calcified.

Third, plankton tow and sediment trap studies have found that the depth habitats, seasonal abundances, and carbon and oxygen isotope signatures of the pink and white varieties of *G. ruber* sometimes differ from each other (Deuser and Ross, 1989; Deuser et al., 1981; Kuroyanagi et al., 2008; Tedesco and Thunell, 2003). However, B/Ca values determined from pink and white *G. ruber* specimens in Ni et al. (2007) generally agree within measurement uncertainty. The white variety of *G. ruber* was not present in the waters off Puerto Rico during our 2010 field season, therefore we could not compare B/Ca of pink and white specimens in culture experiments.

Finally, B/Ca predictions based on culture calibrations may not match core-top B/Ca if there are additional controls that have not yet been identified in culture, or if the parameter $[\text{B}(\text{OH})_4^-]/\text{DIC}$ (similar to $[\text{B}(\text{OH})_4^-]/[\text{HCO}_3^-]$) is not an accurate approximation of the carbonate system’s influence on B incorporation.

4.7. Future proxy application and development

Culture experiments have provided new insight into controls on B incorporation, but can this study’s calibrations be useful to

paleoceanographers? Here, we discuss some uncertainties and recommend directions for future proxy development and application.

In our temperature and salinity experiments, foraminifers experienced conditions that fall within the natural range of modern surface ocean variability. In contrast, our carbonate system experiments subjected foraminifers to pH and DIC extremes that exceed natural modern ranges (Table 1). As previously discussed, it was necessary to grow foraminifers under these extreme conditions in order to distinguish the influence of separate carbonate system controls on B/Ca. Now that B/Ca has been found to increase with borate and decrease with DIC (or $[\text{HCO}_3^-]$), more experiments are needed to better quantify B/Ca relationships with these parameters within their natural range. In *G. sacculifer* and *G. ruber*, the rate of B/Ca increase with $[\text{B}(\text{OH})_4^-]$ is not constant. With only 3–5 data points, it is difficult to determine whether the relationship is exponential, or subject to a specific sensitivity threshold. A higher-resolution calibration with more points across the natural seawater pH range is needed to answer these questions.

In the meantime, what can be done with the existing calibrations? As discussed in Section 4.1, carbonate system controls need to be further tested and refined. B/Ca may respond to seawater $[\text{B}(\text{OH})_4^-]/[\text{HCO}_3^-]$, $[\text{B}(\text{OH})_4^-]/\text{DIC}$, or some variation of these. In theory, if total dissolved boron (B_T) and DIC are known in addition to temperature and salinity, $[\text{B}(\text{OH})_4^-]/[\text{HCO}_3^-]$ may be used to estimate seawater pH (e.g., Tripathi et al., 2009; Yu et al., 2007). However, lack of discernible B/Ca sensitivity to temperature in our culture experiments suggests that such temperature-dependent K_D calibrations may need to be revisited (see also Allen et al., 2012).

For the sake of example, if we assume the $[\text{B}(\text{OH})_4^-]/\text{DIC}$ equations are accurate, then seawater $[\text{B}(\text{OH})_4^-]$ concentrations can be estimated from B/Ca and DIC:

$$[\text{B}(\text{OH})_4^-] = \text{DIC} \times (B/\text{Ca} - b)/m \quad (9)$$

and seawater DIC can be estimated if both $[\text{B}(\text{OH})_4^-]$ and B/Ca are known:

$$\text{DIC} = [\text{B}(\text{OH})_4^-] \times m/(B/\text{Ca} - b) \quad (10)$$

However, due to the limited number of data points used in each calibration, uncertainties regarding the ability of these calibrations to predict DIC or $[\text{B}(\text{OH})_4^-]$ are large. The 95%

prediction bands for Eq. (9) span $\sim 100 \mu\text{mol kg}^{-1} [\text{B}(\text{OH})_4^-]$. If the uncertainty of an individual sample ranges from 50–150 $\mu\text{mol} [\text{B}(\text{OH})_4^-]$ per kg seawater, this corresponds to a 0.6 unit range in estimated pH, roughly 3 times greater than the surface ocean pH change between glacial and interglacial periods (Hönisch and Hemming, 2005). Although the correlations between B/Ca and both $[\text{B}(\text{OH})_4^-]/\text{DIC}$ and $[\text{B}(\text{OH})_4^-]/[\text{HCO}_3^-]$ appear promising ($R^2 > 0.75$), the low number of calibration points translate into large predictive uncertainties. For example, with our three *G. ruber* points we cannot yet say at a 90% confidence level whether the fit coefficients in Eq. (4) are positive or negative. More data are needed to improve the predictive power of these calibrations.

It is also important to note that the range of DIC in the *G. sacculifer* dataset (1046–4196 $\mu\text{mol kg}^{-1}$) is much larger than in the *G. ruber* and *O. universa* pH-experiments (in which DIC was constant at 2034 ± 30 and 1986 ± 3 , respectively). This means that the *G. ruber* and *O. universa* calibrations may not be applicable to systems or time periods when DIC was significantly different from these mean experimental DIC values. High- and low-DIC experiments with these species are needed in order to test calibration accuracy.

5. Conclusions

Culture experiments with live specimens of *G. sacculifer* indicate that B/Ca of foraminiferal calcite depends on the relative abundance of dissolved $\text{B}(\text{OH})_4^-$ and inorganic carbonate species in seawater. B/Ca increases as seawater $[\text{B}(\text{OH})_4^-]$ and pH are raised (while DIC is held constant), but decreases as DIC is raised (while pH and $[\text{B}(\text{OH})_4^-]$ are held constant). This suggests competition between borate and carbonate species for inclusion into the crystal lattice. Fossil foraminiferal calcite B/Ca thus may reflect the relative abundance of these chemical species in ancient seawater. In cultured *G. ruber* and *G. sacculifer*, B/Ca does not increase with temperature, but increases with salinity at rates of 4.5 and 5.1 $\mu\text{mol mol}^{-1}$ per salinity unit, respectively. Normalizing B/Ca data to a constant salinity (e.g., $S=35$) should improve our ability to isolate the carbonate chemistry signal in B/Ca paleorecords and samples from different ocean sites. Final sac chambers amputated from *G. sacculifer* specimens yield lower B/Ca than older chambers from the same specimen, suggesting that the B/Ca composition of gametogenic calcite may be depleted in B compared to the rest of the test.

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Appendix A. Supplementary materials

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.epsl.2012.07.004>.

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