Paleoceanography

Supporting Information for

Calibration of the B/Ca proxy in the planktic foraminifer *Orbulina universa* to Paleocene seawater conditions

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Introduction

This supplementary material includes details on calculations performed in this study, auxiliary culture data, and the methodology for the measurement of foraminiferal shell surface area. Growth rate estimates, individual and average shell weights, and all culture experiment parameters are reported. The sequence of steps for calculating carbonate system variables at the PETM is detailed. We also provide the output for our calibration scenario in which we use measured B/Ca and our new "Paleocene" calibrations to calculate total dissolved inorganic carbon (DIC) across the PETM excursion. Trace element data for a comparison between foraminifers that were terminated prior to gametogenesis versus foraminifers that completed the life cycle are also reported. Finally, we detail the results of an alternate calibration scenario explored in the main text.

S1. Methodology for B/Ca Measurements at the Bristol Isotope Group

In addition to the culture data from 2013 (Santa Catalina Island) and 2015 (Puerto Rico), we also report here a previously unpublished pH calibration for B/Ca in *O. universa* from the Puerto Rico field site for accurate comparison to our data collected there (Table S2, Figure S1). These pH experiments, cultured at Puerto Rico in 2010, were analyzed at the Bristol Isotope Laboratory. Foraminiferal cleaning procedures follow Rae et al. (2011) and Henehan et al. (2015), and are identical to those described in the main text, but with an oxidative cleaning solution of 1% H_2O_2 buffered with 0.1 M NH_4OH, and dissolution in 0.075 M HNO_3. These samples were analyzed on an Element 2 SF-ICP-MS by sample-standard bracketing following Foster (2008), with samples diluted in 0.5 M HNO_3 and matrix-matched to the same Ca concentration as the standards (3 mM Ca). Long term 2 RSD reproducibility of secondary standards, consisting of solutions with similar elemental compositions to dissolved carbonates, is <5 % for B/Ca and <3 % for Mg/Ca. Bracketing standards were calibrated to standards used at Rutgers University, minimizing inter-laboratory bias between these labs.

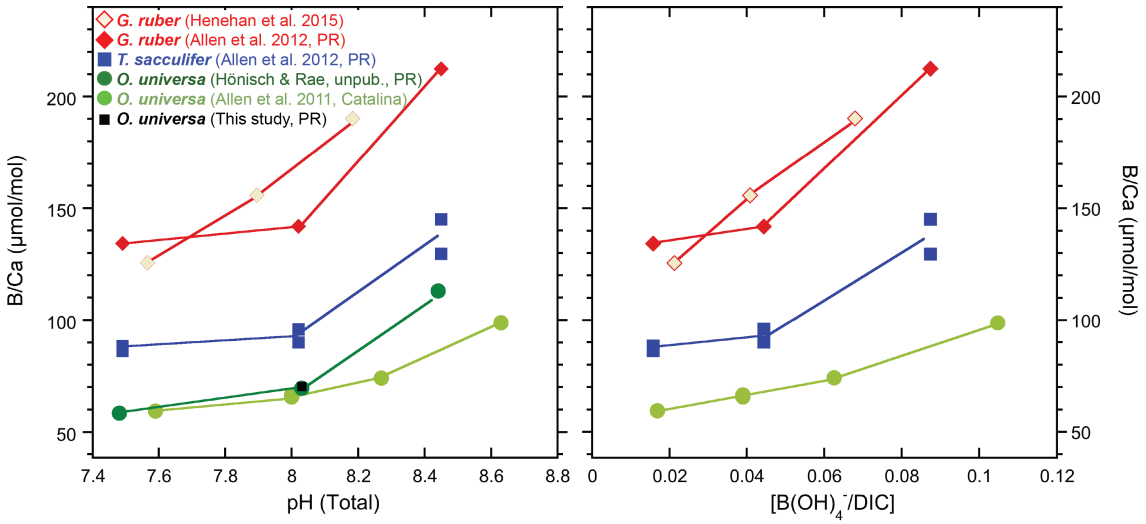

Figure S1. The sensitivity of B/Ca to pH and $[B(OH)_4^-]$/DIC in pH culture experiments from Allen et al. (2011, 2012) and Henehan et al. (2015) and this study. *Globigerinoides ruber* (red) records the highest overall B/Ca values, and shows the highest sensitivity to pH. The ambient data from the Puerto Rico 2015 season (BH6, this study) are shown as black squares and agree well with data of the Puerto Rico 2010 pH calibration, measured at Bristol. The pH calibration for *O. universa* from Puerto Rico is shown in dark green, and appears to have a higher sensitivity than *O. universa* from Catalina, though this is mostly driven by by the high pH experiment.

S2. Estimation of Growth Rate from Brunauer-Emmett-Teller Measurements

We estimated average foraminifer shell growth rates for each of our foraminifers in culture from weight and duration of sphere thickening data. To find area-normalized

growth rates for comparison to inorganic calcites, however, we need to estimate the surface area of each spherical shell in addition to growth rates in µg/day. To estimate shell surface area, we use Brunauer-Emmett-Teller (BET) measurements of pooled fossil *O. universa* shell samples in the 425-515, 515-600, and 600-865 µm size fractions of Fish et al. (in prep.) to estimate the approximate corresponding surface area of cultured foraminifer shells in these given size fractions. Samples were collected from a sub-core of R/V SONNE SO164-17-1 box core within the Florida Straits (Lat: 24°04,93N Long: 80°52,89W, water depth 952 m). Core-top samples were washed, sieved, and picked for *O. universa* in the above size fractions. BET measurements were made at USC on samples of pooled foraminifer shells using a Micromeritics ASAP 2010 instrument and Kr as the analysis gas (de Kanel and Morse, 1979; Subhas et al., 2015). The number of individuals included in each sample ranged from 110-380, depending on the size fraction and the associated surface area based on the required amount of ≥0.05 m^2 surface area per sample. Surface area measurements for each pooled sample yield results in m^2/g. To find the surface area of an average individual shell in each BET sample, we first divided the weight of the BET sample by the number of individuals included to find the average shell weight (Table S1). Then, the average shell weight was multiplied by the average BET-derived surface area in m^2/g to find the average individual shell surface area in this size fraction. We then applied these averages to our cultured foraminifer shells based on their final measured size. For example, a cultured foraminifer with a diameter of 500 µm was assumed to have the average surface area of a foraminifer from the 425-515 µm size fraction BET sample.

S3. Effects of Using Pre-Gametogenic (Terminated) Foraminifers and Implications for Catalina Low Light Experiments

Out of 19 total foraminifers in our low light experimental sample from Catalina Island we included one foraminifer that was terminated prior to gametogenesis. To assess the trace element signature of pre-gametogenic foraminifers, we conducted a paired trace element analysis of gametogenic and pre-gametogenic foraminifera from the same experiment in Puerto Rico (BH7, Table S4). We found that both B/Ca and Mg/Ca are significantly elevated in pre-gametogenic foraminifers compared to foraminifers that underwent gametogenesis; B/Ca was found to be higher by ~15 µmol/mol (23%), while Mg/Ca was elevated by ~4 mmol/mol (42%, Figure S2). Given the increase observed in both elemental ratios, this may suggest that gametogenic calcite has a lower concentration of impurities; however, a consistent decrease in B/Ca and Mg/Ca towards the outside of the shell is not observed in laser ablation profiles (e.g. Holland et al. 2017). Nevertheless, this implies that combining gametogenic and pre-gametogenic foraminifera in trace elemental analyses can significantly bias trace element results, with the effect being larger for Mg/Ca than B/Ca.

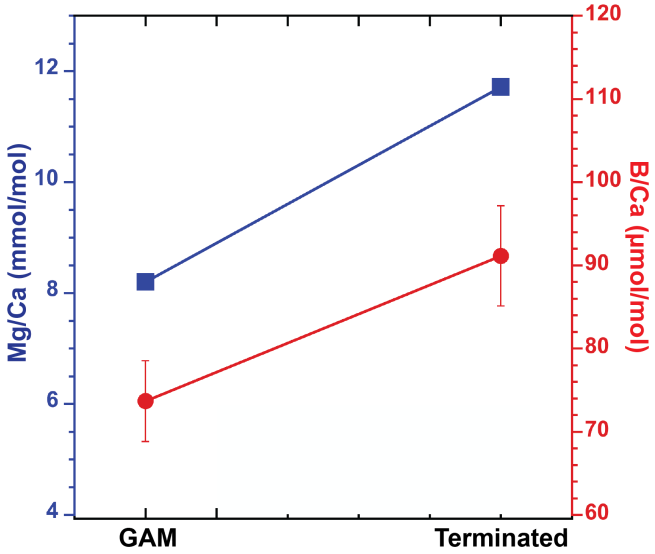

Figure S2. The effect of using terminated versus gametogenic foraminifers in trace element analyses. Foraminifers that were terminated prior to gametogenesis had significantly higher B/Ca (red) and Mg/Ca (blue) than GAM foraminifers from the same experiment. Error bars are 2σ. Errors on Mg/Ca (0.8%) are too small to be observed in this plot.

Based on the pre-cleaning weight of the one pre-gametogenic foraminifer we included, it could at maximum have contributed 9% of the total shell sample weight. However, given that pre-gametogenic shells include a much greater amount of organic matter than shells that have undergone gametogenesis, it is likely that the actual shell weight contribution was much lower (<5%). Nevertheless, we must consider whether this contribution could have biased our results. If pre-gametogenic foraminiferal calcite were a significant contributor to the bulk signal, both B/Ca and Mg/Ca could be elevated above the true value of gametogenic specimens. This is difficult to determine for B/Ca in low light experiments, as we anticipated that low light conditions would decrease B/Ca, while a contribution of pre-gametogenic foraminiferal calcite would increase it, effectively canceling out the low light signal. In contrast, we would expect that both low light conditions and contributions of pre-gametogenic foraminferal calcite would increase Mg/Ca; thus, a pre-gametogenic calcite signature would amplify the low light signal. However, we do not see any increase in shell Mg/Ca in low light experiments on Santa Catalina Island compared to Mg/Ca from the ambient high light experiments of Allen et al. (2011, reported in Allen et al. 2016, Figure 7a, Table 2). Consequently, this result supports the observation from location specific B/Ca calibrations that the sensitivity of foraminiferal symbiont photosynthesis to low light is diminished on Santa Catalina Island (see main text), and that pre-gametogenic foraminiferal calcite was not a significant major contributor to our bulk shell signal.

S4. Calculating Calibration Line Parameters & Uncertainty

All linear fits in this study were calculated using the York_Fit.m script in MatLab (Travis Wiens 2010, from York et al. 2004). Uncertainty on calibration slopes and intercepts were calculated as follows:

1σ uncertainty in seawater-$[B(OH)_4^-]$ and DIC calculated in CO2sys.m (see eq. 3 in the manuscript) was used to calculate 1σ uncertainty in the $[B(OH)_4^-]/DIC$ ratio for each experiment according to the following error propagation equation:

$$\text{Eq. S1} \quad 1\sigma_{[B(OH)_4^-/DIC]} = [B(OH)_4^-/DIC] * \sqrt{\left(\frac{\sigma_{Borate}}{Borate}\right)^2 + \left(\frac{\sigma_{DIC}}{DIC}\right)^2}$$

1σ values for $[B(OH)_4^-]/DIC$ and measured B/Ca were entered into the York_Fit.m script to calculate resultant 1σ uncertainties on calibration slope and intercept. 2σ uncertainties are reported in Table 2.

S5. Shifting Calibration Curves through pre-PETM Conditions

To apply our calibrations to the B/Ca data measured at the PETM in *M. velascoensis* (Penman et al. 2014), we calculated new intercepts for each calibration to pass through the pre-PETM average B/Ca and $[B(OH)_4^-]/DIC$ conditions (Table 3). Average measured pre-PETM B/Ca was 70.4 μmol/mol (Penman et al. 2014). We calculate average pre-PETM $[B(OH)_4^-]/DIC$ by parameterizing the CO2SYS.m script with the $\delta^{11}B$-derived pre-event pH data (assuming a pre-PETM pH of 7.8) and T and S trajectories of Penman et al. (2014), assuming DIC was constant at 2000 μmol kg^{-1}. $[B(OH)_4^-]/DIC$ values for all pre-PETM data were averaged to find the pre-event condition ($[B(OH)_4^-]/DIC$=0.032). We then passed our calibrations through the average pre-PETM condition (70.4 μmol/mol B/Ca, 0.032 $[B(OH)_4^-]/DIC$) to find the intercept appropriate for application to the Paleocene data.

S6. Application to the PETM

1. Predicted B/Ca at the PETM

In Section 4.4, we predict the B/Ca excursion that we expect to result from the pH excursion of Penman et al. (2014) given the B/Ca vs. pH calibrations of Allen et al. (2011, modern seawater) and this study (simulated Paleocene seawater). We use $\delta^{11}B$-derived pH estimates across the PETM to calculate the associated change in $[B(OH)_4^-]/DIC$ values across the event using the CO2SYS.m script in Matlab, modified for the parameterization of $[B]_T$, [Mg], and [Ca]. In the CO2SYS.m script, two carbonate system input parameters, such as total DIC, alkalinity, or pH, are needed. In our calculations, we first assume that DIC remained constant at 2000 μmol kg^{-1} and that pH is the only parameter that changed (decreased) across the PETM. Temperature and salinity were parameterized according to Penman et al. (2014), where temperature estimates were

derived from Mg/Ca measurements on *M. velascoensis*. [Mg] and [Ca] were set at 30 and 20 mmol/kg, (0.6x and 2x modern values), respectively (Table S6).

To estimate uncertainty on calculated $[B(OH)_4^-]/DIC$ values resulting from uncertainties in reconstructed pH, we used the absolute value of the uncertainty associated the lower pH bound. This is because the uncertainty in pH estimates is non-linear, and the lower uncertainty is thus a larger, most conservative estimate. We ran the mean pH value + this 1σ uncertainty in pH estimates through the same CO2SYS.m script and multiplied by two to find the resultant 2σ uncertainty on $[B(OH)_4^-]/DIC$. To predict B/Ca values, we applied both the modern (Allen et al. 2011) and "Paleocene" calibrations (this study, below) (Figure 8, Figure S5):

$$Eq.S2 \quad B/Ca\ (\frac{\mu mol}{mol}) = 1147 \times \left(\frac{B(OH)_4^-\ (\frac{\mu mol}{kg})}{\boldsymbol{DIC}\ (\frac{\mu mol}{kg})} \right) + 33.5$$

As noted in the main text, using the $[B(OH)_4^-]/DIC$ ratio over the $[B(OH)_4^-]/HCO_3^-$ ratio does not alter our interpretation, as the calibration sensitivity is the same (Figure S3). This is because DIC is primarily composed of HCO_3^- in our experimental pH range.

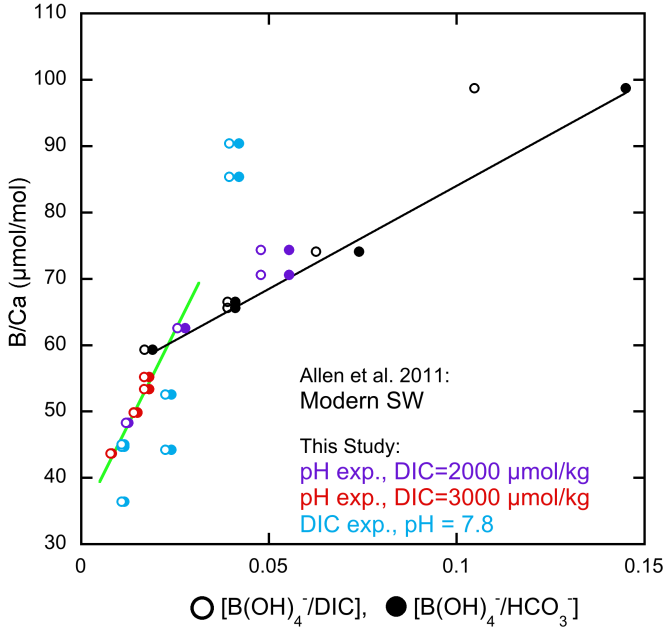

Figure S3. Paleocene and Modern B/Ca calibrations vs. $[B(OH)_4^-]/DIC$ (open symbols) and $[B(OH)_4^-/HCO_3^-]$ (closed symbols). Paleocene data from this study is shown in colored symbols. The assumption that $[B(OH)_4^-]/DIC$ is the controlling parameter on B/Ca instead of $[B(OH)_4^-/HCO_3^-]$ does not change the observed increase in sensitivity at low pH and high DIC in Paleocene experiments. Linear regression lines for modern pH experiments (black) and Paleocene low pH/high DIC experiments (green) are shown.

Uncertainty in predicted B/Ca was estimated in MatLab by creating random variables of N=1000 with mean and standard deviation of calculated $[B(OH)_4^-]/DIC$ (as above), calibration slope, and intercept. The uncertainty associated with the intercept that we use in our calibration was the 1σ value of pre-PETM B/Ca measurements, which is what we used to calculate the intercept (average = 70.4 +/- 1.4, see section E above). Each random variable was propagated through the above calculation to find the resultant 2σ uncertainty in predicted B/Ca due to the uncertainty associated with each parameter. To find total uncertainty for each predicted B/Ca point, an error propagation was performed according to the following equation, to combine uncertainties in calculated B/Ca due to each input parameter uncertainty:

$$Eq.S3 \qquad 2\sigma_{Predicted\ B/Ca} = \sqrt{(2\sigma)^2_{[\frac{borate}{DIC}]} + (2\sigma)^2_{intercept} + (2\sigma)^2_{slope}}$$

2. Calculating DIC at the PETM

We use our "Paleocene" calibration for B/Ca versus both pH and DIC at $[B(OH)_4^-]/DIC<0.03$ to calculate the DIC increase required to explain the full B/Ca excursion at the PETM (Eq. S2). To solve for DIC, we need: 1) Estimates of $[B(OH)_4^-]$, and 2) measured B/Ca data at the same time points. Data for this calculation come from Penman et al. (2014), where B/Ca was measured at site 1209 in *M. velascoensis,* and a $\delta^{11}B$ pH reconstruction from the same site gives us $[B(OH)_4^-]$ estimates (calculated in CO2SYS.m as above).

However, B/Ca and $\delta^{11}B$ were not measured on the same samples, so we must interpolate B/Ca data to the time points when $\delta^{11}B$ was measured. To do this, we firstly created a 5-point running mean of measured B/Ca values in *M. velascoensis* (Penman et al. 2014, Table S6, Figure S4). Where multiple measurements were made at a single time point, measurements from that time interval were averaged before creating the running mean. To preserve the discrete interval of the PETM from averaging, we created two running mean sections, one each for the pre- and post-CIE, each using the CIE as the average starting point. We then linearly interpolated our 5-point running mean values to the time points of $\delta^{11}B$ measurements (Figure S4).

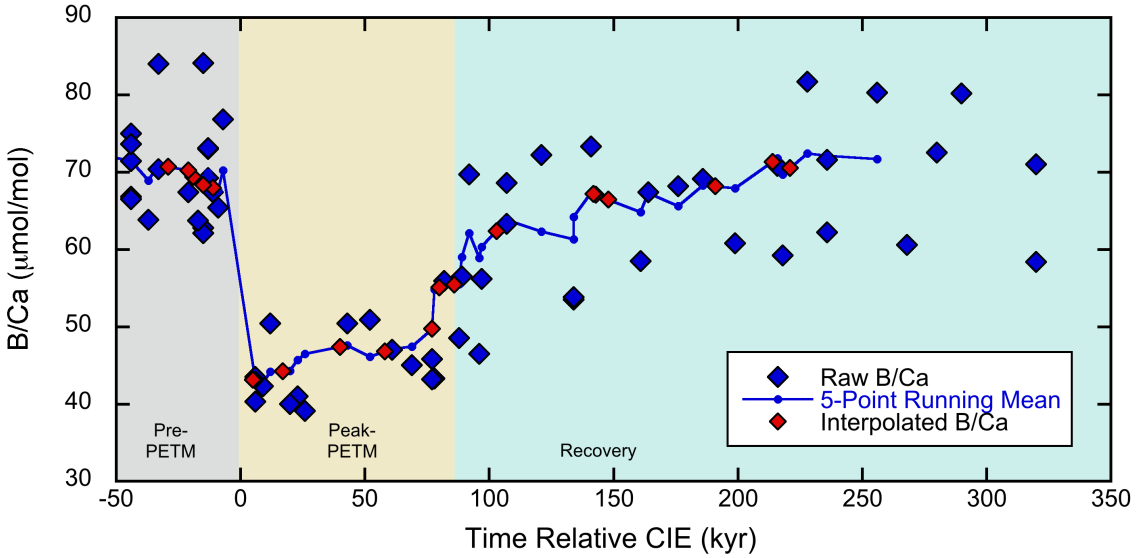

Figure S4. 5-point running mean (blue dots & line) of measured B/Ca from Penman et al. (2014, blue diamonds) and linear interpolation to time points where $\delta^{11}B$ was measured (red diamonds).

We then calculate DIC by re-arranging our calibration equation as follows:

$$Eq.S4 \quad DIC\ (\frac{\mu mol}{kg}) = \frac{B(OH)_4^-(\frac{\mu mol}{kg})}{(B/Ca\ (\frac{\mu mol}{mol}) - 33.5)/1147}$$

While our experimental conditions only extended to 4000 μmol kg^{-1} DIC, we assume that our calibrations extend linearly beyond this point to calculate maximum DIC.

To calculate $\Omega_{calcite}$ associated with this calculated DIC excursion, we used the CO2SYS.m script to solve for the carbonate system at each time point (Table S6). We used the same T, S, and pH parameterizations as in the descriptions above, and varied DIC according to our calculated values.

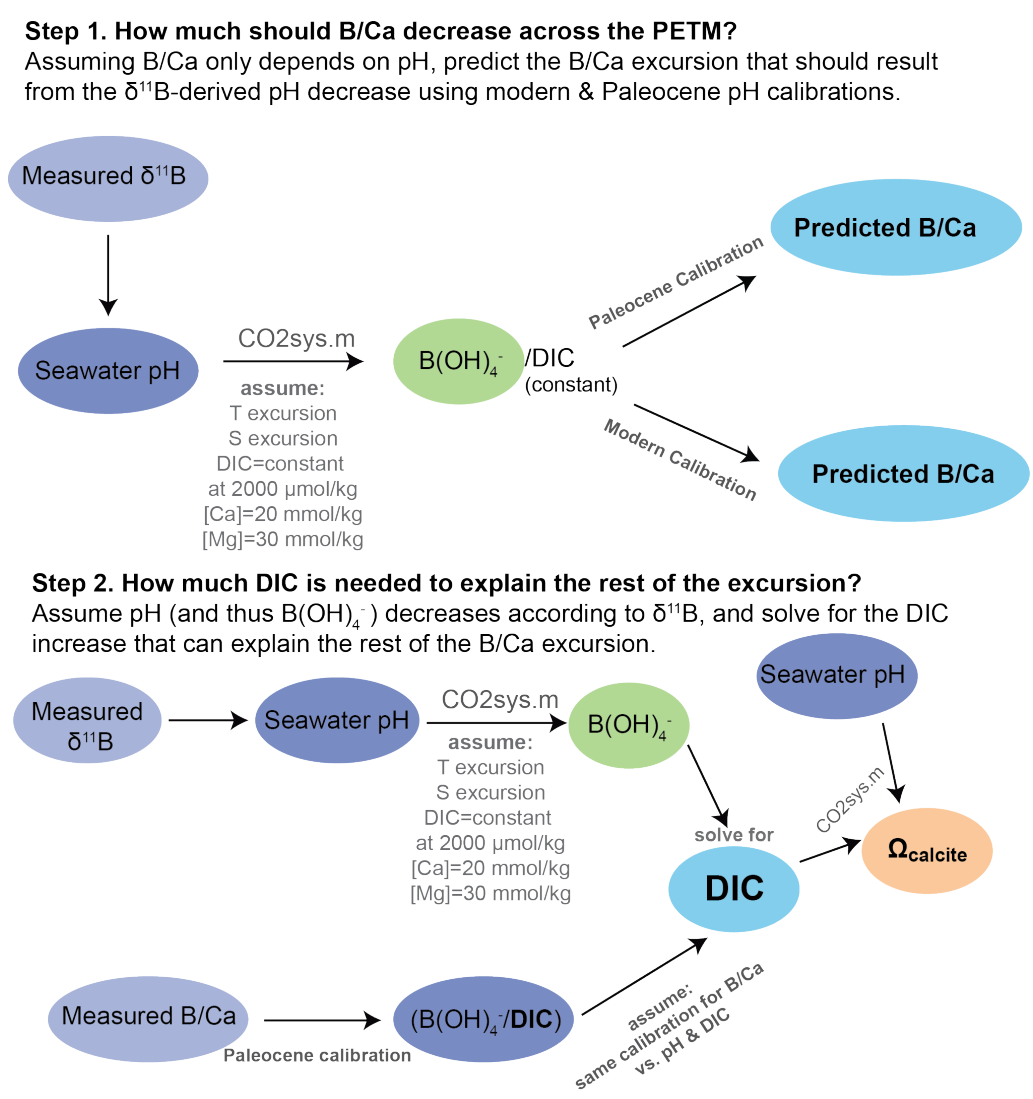

Figure S5. Schematic for PETM carbonate chemistry calculations. The calibration or assumptions used in each calculation step are shown in grey. Steps where the CO2sys.m Matlab script was used to calculate the carbonate system are noted.

3. Alternate Calibration Scenario

In our highest DIC experiment, two replicates show good reproducibility while one is significantly lower by about 10 µmol/mol (Figure 3, Figure S6, see main text for details). We investigate the alternate calibration scenario in which we include this lower replicate in our linear regression. Including this data point in our regression increases the sensitivity of our calibration (slope m= 1495 ± 302 versus 1147 ± 283), though the two are within 2σ uncertainty of each other. We explore the implications of using the more sensitive calibration for application to PETM data in Figure S7. When the one low replicate is included in the calibration, it decreases the reconstructed ΔDIC excursion to ~+1000 μmol kg^{-1}, compared to the ~+2500 μmol kg^{-1} increase observed when the replicate is not included. In addition, calculated $\Omega_{calcite}$ no longer significantly increases in this scenario, which is more consistent with the notion of PETM surface ocean acidification (Penman et al. 2014) than a large increase in $\Omega_{calcite}$.

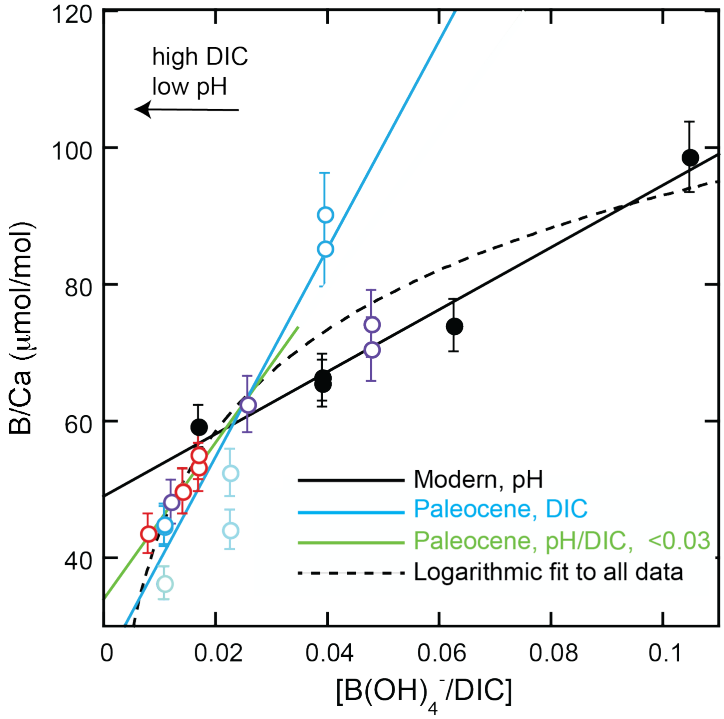

Figure S6. Scenarios for calibration fits to modern and Paleocene B/Ca culture data. The modern seawater data of Allen et al. (2011) are shown in black. pH experiments at constant DIC= 2000 μmol kg^{-1} (purple circles) and 3000 μmol kg^{-1} (red circles) are shown as in main text Figure 3. DIC experiments (at constant pH_{Tot}=7.8) are shown in blue with a linear fit added. The green line is a linear fit to all data where [$B(OH)_4^-$]/DIC

<0.03, excluding the DIC=2063 μmol kg^{-1} experiment and the DIC=4000 μmol kg^{-1} replicate that is lower (light blue circles). A logarithmic fit to all of the data is shown in the dashed line. While the fit appears to explain the data when [B(OH)$_4^-$]/DIC<0.03, at [B(OH)$_4^-$]/DIC>0.03 pH and DIC experiments diverge, precluding a simple singular fit to the data.

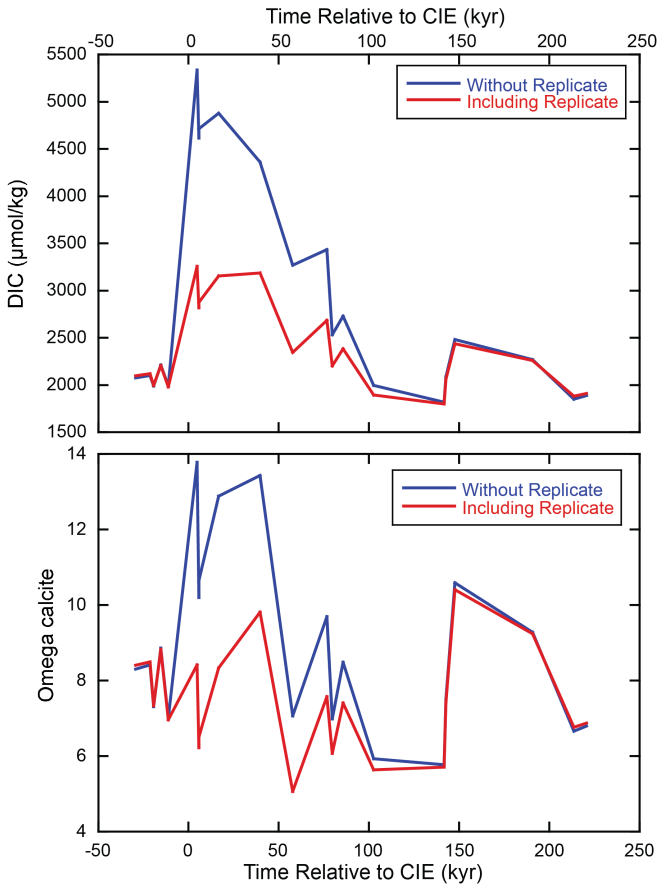

Figure S7. Reconstructed DIC and $\Omega_{calcite}$ using two formulations of the Paleocene B/Ca calibration. The blue line shows the case where the lower replicate from our highest DIC experiment (LH5) is not included. The red line shows the reconstructions when the replicate is included, and calibration sensitivity drastically increases.

This result illustrates that by calibrating only one foraminifera species for the "Paleocene", the calibration uncertainties on reconstructing ΔDIC or $\Omega_{calcite}$ at the PETM are rather large for quantitative application; a small change in calibration formulation has the capacity to change the overall interpretation of the record entirely. Consequently, calibration of other symbiont-bearing species is necessary to reduce the uncertainty on PETM ocean acidification estimates, and to further confirm the effect we observe at low [B(OH)$_4^-$]/DIC in the one species (*O. universa*) that we studied herein.

Supplementary Tables.

Table S1. Brunauer-Emmett-Teller Measurements of pooled fossil *O. universa* shell surface area (SSA).

Size Fraction (µm)	Avg. SSA (m^2/g)	Number of shells included	Average Individual Shell Weight (mg)	Average Individual SSA (m^2)
425 - 515	4.3	380	0.037	0.000158
515 - 600	4.3	110	0.055	0.000235
600 - 865	4.7	216	0.078	0.000369

Table S4. B/Ca and Mg/Ca data of foraminifers at different life cycle states.

Sample	Life cycle state	B/Ca µmol/mol	2σ	Mg/Ca mmol/mol	2σ
BH7a	Not Gametogenic	91	6	11.71	0.09
BH7b	Gametogenic	74	5	8.20	0.07
% Change		**24**		**43**	

Table S5. p-values for the null hypothesis that shell weights and growth rate are uncorrelated with experimental parameters.

Variable	p value	
	Shell Wt. (µg)	µg/day
[Ca]	0.25	0.88
[B]t	0.16	0.79
DIC	0.009*	0.28
pH	0.18	0.18

*denotes significance at a 90% confidence level.