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Abstract: The boron isotope-pH proxy, applied to mixed-layer planktic foraminifera, has great potential for estimating past CO₂ levels, which in turn is crucial to advance our understanding of how this greenhouse gas influences Earth's climate. Previous culture experiments have shown that, although the boron isotopic compositions of various planktic foraminifera are pH dependent, they do not agree with the aqueous geochemical basis of the proxy. Here we outline the results of culture experiments on *Globigerinoides ruber* (white) across a range of pH (~7.5 - 8.2) and analysed via multicollector inductively-coupled plasma mass spectrometry (MC-ICPMS), and compare these data to core-top and sediment-trap samples to derive a robust new species-specific boron isotope-pH calibration. Consistent with earlier culture studies, we show a reduced pH dependency of the boron isotopic composition of symbiont-bearing planktonic foraminifera compared to borate ion in seawater. We also present evidence for a size fraction effect in the $\delta^{11}\text{B}$ of *G. ruber*. Finally, we reconstruct atmospheric CO₂ concentrations over the last deglacial using our new calibration at two equatorial sites, ODP Site 999A and Site GeoB1523-1. These data provide further grounding for the application of the boron isotope-pH proxy in reconstructions of past atmospheric CO₂ levels.

1 **Calibration of the Boron Isotope Proxy in the Planktonic Foraminifera**
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ABSTRACT

The boron isotope-pH proxy, applied to mixed-layer planktic foraminifera, has great potential for estimating past CO₂ levels, which in turn is crucial to advance our understanding of how this greenhouse gas influences Earth's climate. Previous culture experiments have shown that, although the boron isotopic compositions of various planktic foraminifera are pH dependent, they do not agree with the aqueous geochemical basis of the proxy. Here we outline the results of culture experiments on *Globigerinoides ruber* (white) across a range of pH (~7.5 - 8.2) and analysed via multicollector inductively-coupled plasma mass spectrometry (MC-ICPMS), and compare these data to core-top and sediment-trap samples to derive a robust new species-specific boron isotope-pH calibration. Consistent with earlier culture studies, we show a reduced pH dependency of the boron isotopic composition of symbiont-bearing planktonic foraminifera compared to borate ion in seawater. We also present evidence for a size fraction effect in the $\delta^{11}\text{B}$ of *G. ruber*. Finally, we reconstruct atmospheric CO₂ concentrations over the last deglacial using our new calibration at two equatorial sites, ODP Site 999A and Site GeoB1523-1. These data provide further grounding for the application of the boron isotope-pH proxy in reconstructions of past atmospheric CO₂ levels.

KEYWORDS:

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54 Boron isotopes *Globigerinoides ruber* MC-ICPMS Culture

55 calibration pCO₂ reconstruction planktic foraminifera

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58 1. Introduction

59

60 1.1 *The Boron Isotope-pH Proxy*

61

62 The use of boron isotopes in surface-dwelling planktic foraminifera to
63 reconstruct ocean pH, and hence past levels of atmospheric CO₂ (expressed
64 as partial pressure: pCO₂), offers great promise (Foster, 2008; Hönisch and
65 Hemming, 2005; Hönisch et al., 2009; Palmer et al., 2010; Pearson and
66 Palmer, 2000; Pearson et al., 2009; e.g. Sanyal et al., 1995). However,
67 because of the range of offsets from the boron isotope composition of ambient
68 borate ion that has been reported to date in planktonic foraminifera (Sanyal et
69 al., 1996, 2001; Hönisch et al., 2003; Hönisch and Hemming, 2004; Foster,
70 2008), application of the proxy beyond those extant species with species-
71 specific calibrations requires further assumptions to be made. Clearly, then, it
72 is important to extend the range of planktonic foraminiferal species for which
73 empirical calibrations exist, and in doing so better understand exactly how the
74 observed offsets from borate ion, known collectively as “vital effects”, arise.

75

76 The boron isotope-pH proxy has a well-understood foundation in
77 inorganic chemistry that sets it apart from more empirically-derived proxies

(e.g. Li/Ca) and that, providing biological interferences are understood, should permit greater confidence in boron-based palaeo-pH and -pCO₂ estimates. This chemical basis, summarised briefly here, is dealt with in more detail elsewhere (Foster, 2008; Hemming and Hanson, 1992; Hemming and Hönisch, 2007; Pagani et al., 2005; Rae et al., 2011; Sanyal et al., 2000; e.g. Vengosh et al., 1991).

Boron has two stable isotopes, ¹¹B and ¹⁰B (approximately 80% and 20% of total B respectively), with isotopic composition expressed in delta notation as δ¹¹B relative to NIST-SRM 951 boric acid (Catanzaro et al., 1970), where

$$(1) \quad \delta^{11}B = \left[\frac{{}^{11}B/{}^{10}B_{sample}}{{}^{11}B/{}^{10}B_{NIST-SRM\ 951}} - 1 \right] \times 1000$$

Boron is present in seawater almost exclusively as either tetrahedrally-coordinated borate ion, B(OH)₄⁻, or trigonally-coordinated boric acid, B(OH)₃, with the relative abundances of these compounds dependent on pH (see Fig. 1a). Equilibration is rapid (Zeebe et al., 2001) and described by the following disassociation equation

Associated with this equilibration is an isotopic fractionation, described as

where the equilibrium constant ¹¹⁻¹⁰K_B is

$$(4) \quad {}^{11-10}K_B = \frac{[{}^{11}B(OH)_3] \times [{}^{10}B(OH)_4^-]}{[{}^{10}B(OH)_3] \times [{}^{11}B(OH)_4^-]}$$

This constant is often termed α_{3-4} or α_B (e.g. Rae et al., 2011) but, following Klochko et al. (2006), we use $^{11-10}K_B$ here. This equilibrium constant was derived experimentally by Klochko et al. (2006) as 1.0272 ± 0.0006 (in seawater and at 25 °C). This value is in-keeping with the results of numerous independent theoretical studies (e.g. Oi, 2000; Liu and Tossell, 2005; Zeebe, 2005; Rustad and Bylaska, 2007) advocating values in the range of 1.025 to 1.035. Given a value for $^{11-10}K_B$, the isotopic composition of both boron species in seawater varies predictably with pH (see Fig. 1b). Correspondingly, pH can be calculated from the $\delta^{11}B$ of either boron species, for instance for $\delta^{11}B$ of borate as:

$$(5) \quad pH = pK_B^* - \log \left[-\frac{\delta^{11}B_{sw} - \delta^{11}B_{borate}}{\delta^{11}B_{sw} - (^{11-10}K_B \times \delta^{11}B_{borate}) - 1000(^{11-10}K_B - 1)} \right]$$

where pK_B^* is the disassociation constant for boric acid at *in situ* temperature, salinity and pressure (most commonly calculated as per Dickson, 1990). $\delta^{11}B_{sw}$ is the isotopic composition of seawater (39.61 ‰; Foster et al. 2010), and $^{11-10}K_B$ is 1.0272 ± 0.0006 (Klochko et al. 2006). Since only the charged borate ion is thought to be incorporated into biogenic calcite (Hemming and Hanson, 1992; Hemming et al., 1998), the boron isotopic composition of $CaCO_3$ can be used to calculate ocean pH (as $\delta^{11}B_{borate}$ in Eq. 5 above) provided any vital effects have been accounted for.

1.2 Calibration attempts to date

Culture studies are an important tool in calibrating foraminifera-based proxies, allowing for manipulation of growth conditions beyond the range seen

124 in the modern oceans (e.g. Allen et al., 2011). In the case of the boron
125 isotope-pH proxy, attempts have been made to calibrate the symbiont-bearing
126 foraminifera *Orbulina universa* (Sanyal et al., 1996) and *Globigerinoides*
127 *sacculifer* (Sanyal et al., 2001) across a range of pH (7.6 to 9). These culture
128 studies confirmed that the $\delta^{11}\text{B}$ of planktic foraminiferal calcite is strongly
129 dependent on pH, but also describe a weaker sensitivity to pH in foraminiferal
130 $\delta^{11}\text{B}_{\text{CaCO}_3}$ compared to $\delta^{11}\text{B}_{\text{borate}}$ (Figure 2a). While these studies were
131 pioneering, some uncertainty still remains in the robustness of these
132 relationships. For instance, some carbonate system parameters during
133 culture in these studies are relatively poorly constrained (e.g. pH only
134 determined using NBS-buffer-calibrated electrodes, with the magnitude of any
135 correction to total scale greatly influencing conclusions to be made: see
136 Supplementary Fig. 1) and some experiments (e.g. Sanyal et al., 2001) were
137 performed at 10 x natural boron concentration, introducing additional
138 uncertainty by increasing the buffering capacity of culture seawater and thus
139 potentially dampening 'vital effects' (Zeebe et al., 2003). Furthermore, limited
140 core-top calibration attempts by Foster (2008) (measured by multicollector
141 inductively coupled plasma mass spectrometry; MC-ICPMS) suggested a
142 stronger pH sensitivity in the $\delta^{11}\text{B}$ of *G. ruber* and *G. sacculifer* (close to that
143 of borate ion), leading to the suggestion that these previous calibrations may
144 have been compromised by analytical issues related to the analytical
145 technique used (negative ion thermal ionisation mass spectrometry; NTIMS;
146 e.g., Foster, 2008; Ni et al., 2010). Therefore, further culture calibrations are
147 required, both to corroborate previously reported pH sensitivities and to
148 extend the applicability of the boron isotope-pH proxy to more species of

foraminifera.

1.3 Presentation of species calibrations

The most common way to present boron isotope data from cultured foraminifera is in terms of the observed $\delta^{11}\text{B}$ -pH relationship (Fig 2a). This, however, leads to difficulties in comparing calibrations generated under differing temperatures and salinities, where the value of pK_B^* differs, introducing the need for normalisation. For example, in Figure 2a, calibration datasets are normalised to our culture conditions (26 °C and 37.2 psu), based on the change in aqueous $\delta^{11}\text{B}_{\text{borate}}$ expected if pK_B^* were altered to reflect these conditions. Similarly, application of these calibrations to open ocean foraminiferal data must include some correction for pK_B^* differences between datasets.

Furthermore, in order to present boron isotope data in terms of the observed $\delta^{11}\text{B}$ -pH relationship, it is necessary to characterise species' $\delta^{11}\text{B}$ -pH relationships by forcing them to fit the general equation for boron isotope-pH calculation (Eq. 5 above). This can be done by incorporation of a constant offset or 'vital effect' termed 'a' into equation (5), such that

$$\delta^{11}\text{B}_{\text{borate}} = \delta^{11}\text{B}_{\text{CaCO}_3} - a .$$

$$(6) \quad pH = pK_B^* - \log \left[- \frac{\delta^{11}\text{B}_{\text{sw}} - (\delta^{11}\text{B}_{\text{CaCO}_3} - a)}{\delta^{11}\text{B}_{\text{sw}} - {}^{11-10}K_B(\delta^{11}\text{B}_{\text{CaCO}_3} - a) - 1000({}^{11-10}K_B - 1)} \right]$$

In addition, the fit of empirical data to an equation of this form can also be optimised by altering the ${}^{11-10}K_B$ constant (Table 1, Hönisch et al., 2007). The addition of an offset 'a' to an aqueous $\delta^{11}\text{B}_{\text{borate}}$ -pH curve (Foster, 2008; as in

Hönisch and Hemming, 2005), however, implies that any such ‘vital effect’ is constant across a range of pH. Past modelling efforts (Zeebe et al., 2003) provide some support for this argument, but it must be noted that no culture calibration performed to date (our own included) shows a constant offset from the $^{11-10}K_B$ of Klochko et al. (2006). In addition, any optimisation of $^{11-10}K_B$ in tandem with an offset ‘a’, as used by Hönisch et al. (2007) to characterise $\delta^{11}B_{\text{carbonate}}$ -pH curves of published empirical data, might be misconstrued as implying $^{11-10}K_B$ is not 1.0272, rather than that these empirical calibrations exhibit pH sensitivities different from the pH sensitivity of $\delta^{11}B_{\text{borate}}$.

Given these difficulties, we instead describe culture calibration data following Foster et al. (2012). Similar to Rollion-Bard and Erez (2010), who plot calculated pH vs. culture pH, this approach involves a linear regression between aqueous $\delta^{11}B_{\text{borate}}$ (at *in situ* conditions) and measured $\delta^{11}B_{\text{CaCO}_3}$ (Fig. 2b). Given measured $\delta^{11}B_{\text{CaCO}_3}$, one can use the appropriate calibration regression to predict the value of ambient $\delta^{11}B_{\text{borate}}$, which may then be entered into the general equation (5) to calculate pH. On such cross-plots, calibration data define straight lines with slopes (given as ‘m’, Table 1) reflecting the difference between the $\delta^{11}B$ -pH sensitivity of $\delta^{11}B_{\text{borate}}$ and $\delta^{11}B_{\text{CaCO}_3}$. Transformations for relevant existing calibrations are listed in Table 1. The slopes and intercepts (given as ‘c’, Table 1) of these lines are independent of pK_B^* (and hence salinity, temperature and pressure), meaning that culture and core top calibrations can be readily compared without reference to *in situ* environmental conditions. In addition, presentation of culture calibrations in this way allows for the plotting of 95% confidence

intervals using York regression, accounting for uncertainty in culture conditions and measurement (Ludwig, 2003; York, 1968). This approach permits better propagation of the uncertainty of culture calibrations into final pH and pCO₂ reconstructions, and confirms the observations of Hönisch et al. (2007) that pH sensitivities seen in published foraminiferal culture calibrations (Sanyal et al. 1996, 2001) are within statistical uncertainty of the pH sensitivity of inorganic CaCO₃ derived by Sanyal et al., (2000). Given also the possibility that species-specific offsets (i.e. the observed range in intercept on Table 1) may be partially due to inconsistencies in absolute N-TIMS measurements between labs (as highlighted by Hönisch et al., 2003; Ni et al., 2010; Rae et al., 2011), further investigation into the species-specificity of these calibrations is required.

2. METHODS

2.1 Culture calibration

2.1.1 Sampling and Culturing

Neanic (typically ~ 250 µm in diameter) specimens of *G. ruber* (white) were towed from depths of <10m in the Gulf of Aqaba between January and March 2010, at the Interuniversity Institute of Eilat, Israel. Individual foraminifera were transferred to seawater collected at the site of plankton towing within 4-6 hours of capture, and kept under saturated light conditions at ~ 23 °C until fully recovered (i.e. floating, with a halo of spines and symbionts). Those that did not recover fully were retained for boron isotope analysis as 'control' tow samples, and used for mass-balance corrections (as discussed later). Since traits used to distinguish the

morphotypes of *G. ruber* (sensu stricto/lato; Wang, 2000) are often poorly developed in immature specimens (Aurahs et al., 2011), no distinction could be drawn between morphotypes in culture. For reference, *G. ruber* sensu lato made up 35-45% of the total identifiable population of *G. ruber* from tows and core-top sediment in the Gulf of Aqaba. However, we see no difference in $\delta^{11}\text{B}$ (or B/Ca) between these morphotypes in core top sediments (see Supplementary Fig. 2).

Recovered foraminifera were transferred to sealed 100 ml Erlenmeyer flasks filled with prepared filtered seawater (see below). Foraminifera were removed daily, fed one newly-hatched *Artemia* nauplius, observed and measured using a Zeiss inverted light microscope. Condition and approximate symbiont density was noted. In an attempt to avoid damage to the foraminifera and increase the low acceptance rates typically seen in cultured *G. ruber* (Spindler et al., 1984), thereby increasing mass gain, *Artemia* were physically incapacitated prior to feeding. Illumination was provided by a metal halide lamp (420 W, Osram™) at levels of 200 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (13h light:11h dark), equivalent to irradiance at 15-20 m depth in the open waters of the northern Gulf of Aqaba (Shaked and Genin, 2006). Culture flasks were kept in water baths at a constant temperature of 26 ± 0.5 °C.

Seawater was prepared in large batches to ensure consistency across all flasks, with surplus for topping up stored in airtight bottles in the dark at ~4 °C. Salinity was reduced from ~40.7 to 37 via addition of de-ionised water.

Following other culturing studies (e.g. Sanyal et al., 1996, 2001), pH was altered by adjusting alkalinity via addition of NaOH or HCl. Culture experiments were carried out at pH 8.174 ± 0.007 , 7.894 ± 0.013 , and 7.554 ± 0.013 (total scale; 2 se, n = 48-67). pH drift in culture flasks was monitored periodically using a pH electrode calibrated to NBS buffers, with individuals from flasks that experienced large pH drift discounted (n = 5). Culture solution was sampled at the beginning of each pH experiment, and a composite sample taken from all flasks at the end of culturing. These water samples were poisoned with 50 μ l saturated HgCl₂ solution and transported for full carbonate system analysis at the UK Ocean Acidification Research Programme (UKOARP) Carbonate Chemistry Facility, at the National Oceanography Centre Southampton (NOCS). Nutrient analyses were also undertaken to ascertain nitrate, nitrite, phosphate and silicate concentrations. NBS-scale pH measurements of these composite samples taken in Eilat were consistently higher than Dissolved Inorganic Carbon (DIC) and Total Alkalinity (TAlk)-derived total scale pH measurements by 0.21 pH units (see Supplementary Fig. 3). As such, in-culture NBS-scale electrode measurements could be used once corrected for this 0.21 pH offset.

After gametogenesis (typically after 6-10 days in culture), empty *G. ruber* tests were removed from culture flasks, rinsed in de-ionised water, dried and weighed.

2.1.2 Mass balance calculations and estimates of growth rate

To correct for the chemistry of the test grown outside of culture, a mass-balance correction was used, following Erez and Luz (1983), Lohmann (1995) and Kısakürek et al. (2008). Dried 'control' samples (i.e. *G. ruber* tests towed at the same time as cultured material) were weighed on a microbalance, photographed and measured using Macnification® imaging software. A size-mass relationship for all towed control samples was calculated, such that initial size measurements (maximum axis multiplied by its perpendicular axis) of foraminifera made immediately prior to culturing could be used to estimate shell mass (Supplementary Fig. 4). While organic matter was not removed prior to weighing, calculation of CaCO₃ mass via ICPMS (as per Villiers et al., 2002) for a subset of samples gave consistent results, suggesting organics did not significantly contribute to dried shell mass. Best fit was via power-type regression, as noted by Kısakürek et al. (2011) for *G. ruber* from Eilat. The relationship is best defined by the equation $mass = 803.65 * (product\ of\ axes)^{1.957}$ (n= 112, R² = 0.75, p < 0.001). Note the exponential of this relationship is the same as that defined by Kısakürek et al. (2011).

The δ¹¹B and B/Ca of control *G. ruber* from each experimental tow (n = 150-200) was then measured (see below for analytical methodology). Assuming cultured individuals began with this δ¹¹B and B/Ca, and using the size-mass relationship to estimate the mass of calcite grown out of culture, a correction can be made and the composition of the foraminifera grown during culture calculated by

$$(7) \quad \delta^{11}B_{culture} = \frac{\delta^{11}B_{measured} - (\delta^{11}B_{controls} \times P_{controls}^B)}{P_{culture}^B}$$

where δ¹¹B_{measured} is the measured boron isotopic composition of the bulk

material, and $\delta^{11}\text{B}_{\text{controls}}$ is the measured boron isotopic composition of 'control' foraminifera towed at the same time as those cultured. P_{controls}^B and P_{culture}^B are the proportions of boron in the bulk material coming from the pre-culture and culture-grown calcite respectively. These are calculated based on the B/Ca ratios in the bulk material and in control specimens, and the proportion of calcite mass grown in culture and out of culture, such that

$$(8) \quad P_{\text{culture}}^B = \frac{B / Ca_{\text{measured}} - (B / Ca_{\text{controls}} \cdot P_{\text{controls}}^{\text{mass}})}{B / Ca_{\text{measured}}}$$

and $P_{\text{controls}}^B = 1 - P_{\text{culture}}^B$. Uncertainty on $\delta^{11}\text{B}_{\text{culture}}$ data was calculated as two standard deviations of 10,000 Monte Carlo simulations accounting for the uncertainty in the size:mass relationship (Supplementary Fig. 4b), B/Ca measurements (5% at 2σ) and $\delta^{11}\text{B}$ measurements (from equation 9 below).

2.2 Core-top calibration

2.2.1 Sampling

To compare the results of our culture calibration with foraminifera grown in natural conditions, we measured *G. ruber* from globally distributed core-top sites from archives at Tübingen, Germany and NIWA, New Zealand. Samples from NIWA were verified as recent by way of ^{14}C -dating (H. Bostock, pers. comm.), while from the Tübingen repository only undisturbed multicore sediment containing Rose Bengal-stained living benthic foraminifera was selected. In addition, material from sediment traps in the Cariaco Basin was used to further test for biases introduced during sedimentation. This array of sites covered as broad a range of *in situ* $\delta^{11}\text{B}_{\text{borate}}$ as possible for regions inhabited by *G. ruber*, and serves to test for regional variations (as seen in

Mg/Ca, Bolton et al., 2011) that might stem from genotype differences (Darling and Wade, 2008). The locations of these core-top and sediment trap sites are shown in Fig. 3 (see also Supplementary Materials). Samples were also taken from a number of size fractions to examine the influence of test size on measured $\delta^{11}\text{B}$.

2.2.2 Carbonate system parameters

pH, temperature and salinity at the sediment trap site (Cariaco Basin, collected January 2007) is interpolated from data from December 2006 and February 2007, downloadable from <http://www.imars.usf.edu/CAR>. pH was estimated for core-top sites using surface water oceanographic data from the GLODAP (Key et al., 2004), CARINA (Key et al., 2010) and Takahashi et al. (2009) compendia (see Supplementary Materials). First, regional salinity-Talk correlations were calculated from surface (< 20 m) GLODAP/CARINA measurements. Applying these correlations, monthly-resolved values of salinity from Takahashi et al. (2009) were converted to monthly TALK estimates. Monthly temperature was also taken from Takahashi et al. (2009). Pre-industrial pCO_2 at each core-top site was estimated by applying ocean-atmosphere ΔpCO_2 from Takahashi et al. (2009) sites (corrected for post-industrial changes in flux with reference to Gloor et al. 2003) to a pre-industrial atmospheric pCO_2 value. Where samples were ^{14}C -dated, the age-appropriate atmospheric pCO_2 value was taken from Lüthi et al. (2008, and references within). Where core-tops were not dated, we assume an average late Holocene (< 4 kyr BP) value of 275 ppm. Combined with approximations of typical local silicate and phosphate concentrations (from GLODAP/CARINA

measurements), monthly estimates of pH were calculated using CO₂sys.m (Van Heuven et al., 2011), and the constants of Lueker et al (2000), Lee et al. (2010) and Dickson (1990).

2.3 Analytical techniques.

2.3.1 Sample cleaning and preparation

Foraminiferal cleaning is largely as described in Rae et al. (2011), in turn based on the approach of Barker et al. (2003). Foraminifera were cracked open between two clean glass slides, ultrasonicated and rinsed repeatedly with MilliQ ultrapure water (18.2 MΩ) to remove clays. Culture, sediment trap and tow samples, in agreement with other culturing studies (e.g. Russell et al. 2004), were subject to intensified oxidative cleaning (3 x 20-30 min treatments of 250 µl 1% H₂O₂ + 0.1 M NH₄OH₄ at 80 °C) to account for the larger organic content. In core-tops, oxidative cleaning was shorter (3 x 5 min) to minimise sample loss. Samples were subject to a brief weak acid leach in 0.0005 M HNO₃ to remove readsorbed contaminants, before dissolution by incremental addition of 0.5 M HNO₃ (typically < 100 µl).

An aliquot (~20 µl) of dissolved sample was taken for elemental analysis, before the remaining sample was buffered and boron separated from matrix using 20 µl-volume columns of Amberlite IRA743 boron-specific resin (Kiss, 1988). Typical column yields for boron are > 99.9%, with an extra acid elution step monitored for boron concentration to check elution efficiency. All sample preparation work and column chemistry was carried out in a dedicated flow hood within an over-pressured clean lab fitted with boron-free HEPA filters,

permitting typical total procedural blanks of < 20 pg.

2.3.2 MC-ICPMS and ICPMS

Samples were measured for boron isotope composition on Thermo Scientific Neptune MC-ICPMS at the University of Bristol (cultures, 'control' tows and GeoB-1523-1), and the University of Southampton (core-tops and sediment trap) according to methods described elsewhere (Rae et al. 2011, Foster 2008). Repeat analysis of a range of carbonate (including *G. ruber*, Supplementary Fig. 5) and boric acid consistency standards (Supplementary Fig. 6) reveals no significant differences between these laboratories.

External reproducibility of the MC-ICPMS $\delta^{11}\text{B}$ method (at 95% confidence, 2σ) is typically < 0.25 ‰ on ~1 mg of CaCO_3 (sample size requirement varies with $[\text{B}]_{\text{calcite}}$, but in the case of *G. ruber* equates to 75-100 tests of diameter 300-355 μm). Following Rae et al. (2011), uncertainty on culture and tow data run at Bristol is calculated as

$$(9) \quad 2\sigma = 1.7 * \exp^{-29[^{11}\text{B}]} + 0.31 * \exp^{-0.75[^{11}\text{B}]}$$

where $[^{11}\text{B}]$ is the intensity of ^{11}B signal in volts. Uncertainty on core-top and sediment trap data is similarly calculated, according to the external reproducibility of repeat analyses of Japanese Geological Survey *Porites* coral standard (JCP; $\delta^{11}\text{B} = 24.3\text{‰}$) at the University of Southampton (Supplementary Fig. 7). This relationship is described by equation 10 below.

$$(10) \quad 2\sigma = 1.87 * \exp^{-20.6[^{11}\text{B}]} + 0.22 * \exp^{-0.43[^{11}\text{B}]}$$

Trace element-calcium ratios (Mg, B, Al, Mn, Ba, Sr, Li, Na, Cd, U, Nd, Fe) were analysed using Thermo Element 2 ICP-MS at the University of

Bristol (cultures, down-core and control tows) and the University of Southampton (core-tops and sediment trap). Here, these data are only used to assess adequacy of clay removal ($\text{Al/Ca} < 100 \mu\text{mol/mol}$; Rae et al., 2011), to generate down-core temperature estimates (Mg/Ca ; see Supplementary Materials), and to correct for boron incorporated outside of culture. A full exploration of trace element data is beyond the scope of this paper, and will follow in later publications.

2.4 Downcore application

2.4.1 Site and species selection

G. ruber white sensu stricto (300-355 μm) were picked from sediments aged 0 to 30 kyr from core site GeoB1523-1, recovered from the Ceara Rise in the western equatorial Atlantic (3.83 °N, -41.62 °E) at a water depth of 3292 m. We use the previously published age model for this site based on $\delta^{18}\text{O}$ (Gingele et al., 2000).

2.4.2 Temperature and salinity estimates

Estimates of sea surface temperature (SST) and salinity (SSS) are required to calculate pH from $\delta^{11}\text{B}$, as these parameters influence pK_B^* . It is important to note, however, that calculated pH is only weakly dependent on these environmental parameters (0.012 pH units per °C; 0.003 pH units per psu). For GeoB1523-1, SST was reconstructed using the Mg/Ca ratio of *G. ruber* measured on an aliquot of the same sample used for isotope measurement and the generic SST calibration ($\text{Mg/Ca} = 0.38 * \exp[\text{SST} * 0.09]$) of Anand et al. (2003). This is preferred over the species-

specific calibration as it better fits our dataset of >20 core-top measurements (Henehan, unpublished data). As with Hönisch and Hemming (2005), palaeosalinity was estimated using the following equation

$$(11) \quad \text{SSS} = \text{SSS}_{\text{modern}} + \Delta_{\text{sea-level}}/3800 * 34.8$$

where $\Delta_{\text{sea-level}}$ is an estimate of sea-level change in metres, 3800 m is the average ocean depth, 34.8 is mean averaged modern ocean salinity, and $\text{SSS}_{\text{modern}}$ is the modern salinity at the site of interest (from GLODAP, Key et al. 2004).

2.4.3 The second carbonate system parameter

Ocean pH is only one variable of the ocean carbonate system, and to determine $[\text{CO}_2]_{\text{aq}}$ and hence pCO_2 using Henry's Law, another variable is required (Zeebe and Wolf-Gladrow, 2001). The second variable chosen, TALK, is calculated from estimated palaeosalinity (Hönisch et al., 2009; as per Palmer and Pearson, 2003), itself derived from equation [11] above, and a TALK vs. SSS relationship defined by modern ocean data from the equatorial Atlantic ($\text{TALK} = \text{SSS} * 61.88 + 162.66$, $R^2 = 0.88$, $p < 0.001$; from GLODAP; Key et al., 2004). However it is important to note that the generated pCO_2 estimate is determined largely by the reconstructed pH, and TALK has little control. For example, given a pH of 8.2 (and SST of 25 °C and salinity 35 psu) drastically increasing TALK from 2400 to 2600 $\mu\text{mol/kg}$ – equivalent to the range modelled by Hönisch et al. (2009) for the last 2 myr – only increases reconstructed pCO_2 by 24 ppm.

Given this reconstruction of TALK and a $\delta^{11}\text{B}$ -derived pH, it is possible to reconstruct the entire carbonate system using $\text{CO}_2\text{sys.m}$ (Van Heuven et

al., 2011). Given the modern disequilibrium of surface waters above GeoB1523-1 ($\Delta p\text{CO}_2$ of 15 to 25 ppm; Takahashi et al. 2009), and a correction factor for the pre-industrial $\Delta p\text{CO}_2$ in this region of 1.33 (derived from Gloor et al. 2003), a correction for disequilibrium of -27 ppm is applied in order to calculate atmospheric CO_2 concentrations from calculated aqueous $p\text{CO}_2$. We apply a conservative approximation of uncertainty on reconstructed aqueous $p\text{CO}_2$ of 29 ppm. This is a quadratic addition of the ranges of uncertainty in reconstructed $p\text{CO}_2$ that are produced via propagation of each input parameter uncertainty in turn, namely the calibration equation (uncertainty as in Table 1 and discussion below), $\delta^{11}\text{B}$ measurement ($\sim \pm 0.2 \text{ ‰}$), and reconstructed salinity ($\pm 1 \text{ psu}$), TALK ($\pm 100 \text{ } \mu\text{mol/kg}$) and temperature ($\pm 1 \text{ }^\circ\text{C}$).

3. RESULTS

3.1 Culturing

Mortality was greatest at our lowest pH, with most individuals surviving to gametogenesis in the two higher pH experiments (54% and 64% at 8.174 and 7.894 pH respectively), but only 40% surviving to gametogenesis at pH 7.554 (compared to pH in the Gulf of Aqaba of ~ 8.075). Note that while these values of survivorship are low compared to cultures of other species, *G. ruber* is notoriously difficult to culture (e.g. Hemleben et al., 1987), and as such the observed high mortality is not unexpected.

Samples grown at low pH (7.554) were typically surrounded by a much denser shroud of symbionts that often entirely obscured the outline of the test

(Fig. 4b). Also, at this low pH individuals often lost most, or all, of their calcitic spines and fed less frequently (every 3-4 days) using short, unsupported, pseudopods. Larger tests across all pH treatments often showed a proliferation of many small and/or kummerform chambers growing in unusual configurations. While unusual within the 300-355 μm size range, it is not uncommon to find such growth patterns in larger *G. ruber* ($> 400 \mu\text{m}$) from core-tops, indicating these forms are not simply a response to culture. At higher pH, chambers were usually visibly more heavily calcified (Fig 4c), and growth rates were higher (see Table 2). At pH 7.554, tests were visibly thinner and often possessed abnormally wide apertures, with evidence in some cases that chambers may have been partially dissolved or reabsorbed during ontogeny (see Fig 4e).

3.2 MC-ICPMS results

The results of boron isotope analyses of culture, core-top, tow, sediment trap and down-core samples (along with the relevant mass-balance corrections in culture experiments) are given in Table 3. These data are plotted in $\delta^{11}\text{B}_{\text{CaCO}_3}$ - $\delta^{11}\text{B}_{\text{borate}}$ space (Fig. 5). York-fit linear regression of culture data produces a $\delta^{11}\text{B}_{\text{CaCO}_3}$ - $\delta^{11}\text{B}_{\text{borate}}$ relationship described by equation 12 below, with uncertainty (2σ , 95%) on this fit, accounting for error in analytical and *in situ* $\delta^{11}\text{B}_{\text{borate}}$ values, shown as a shaded band.

$$(12) \quad \delta^{11}B_{\text{borate}} = (\delta^{11}B_{\text{measured}} - 9.52 \pm 1.51) / (0.60 \pm 0.08)$$

The slope (i.e. pH sensitivity) of this culture calibration is within uncertainty of existing culture and inorganic calibrations (see Table 1), and is significantly

lower than the theoretical pH sensitivity of borate ion in seawater (as indicated by a slope of < 1). For comparison, towed 'control' specimens and coretop specimens are also plotted. We see no discernible effect of geographical location nor sample material type, suggesting that sedimentation or regional differences do not influence recorded $\delta^{11}\text{B}$. Although these data are permissively in agreement with the slope (i.e. $\delta^{11}\text{B}$ -pH sensitivity) of the culture calibration (a York regression of the 300-355 μm size-fraction has a slope of 0.45 ± 0.25 at 95 % confidence), there is a tendency toward lower values of $\delta^{11}\text{B}$ than the culture calibration would predict in size fractions smaller than $\sim 380 \mu\text{m}$, and higher values in larger size fractions (see Fig. 6). As such, and because the spread in pH in the core-top samples alone is too small for a precise $\delta^{11}\text{B}$ -pH calibration, we suggest modification of the culture calibration equation to reflect the size fractions used down-core. Thus for the commonly-used 300-355 μm size fraction a correction of -0.65 (see Fig. 6) should be applied to the intercept value 'c' of the culture $\delta^{11}\text{B}_{\text{CaCO}_3}$ - $\delta^{11}\text{B}_{\text{borate}}$ relationship (equation 12).

$$(13) \quad \delta^{11}B_{\text{borate}} = (\delta^{11}B_{\text{measured}} - 8.87 \pm 1.52) / (0.60 \pm 0.09)$$

Uncertainty on the intercept in this relationship is a quadratic addition of two standard errors of the mean offset from culture in 300-355 μm fraction core-top samples and the uncertainty in the original culture calibration York regression intercept. Similarly, we suggest a correction of -0.83 ‰ for the 250-300 μm size fraction, and -0.16 ‰ for samples of 355-400 μm . For other size fractions the culture calibration may be corrected using the relationship between test size and offset from culture in Fig. 6 (offset = $[0.005 \cdot \text{average size}] - 2.185$, $R^2 = 0.33$), although the narrow size range of *G. ruber* in core-

tops and the large ratio of analytical uncertainty to signal mean that the relationship is not statistically robust, and as such wherever possible we would advocate using size fractions tested here. Indeed, despite the general similarity of size-fraction effects seen across our sample sites (Supplementary Fig. 8), given the limitations of our dataset, we encourage prior verification of the consistency of this size fraction effect at the sites of any future down-core reconstructions.

3.3 $p\text{CO}_2$ reconstruction

The $\delta^{11}\text{B}$ of *G. ruber* from Site GeoB1523-1 are plotted in Figure 7a, along with the *G. ruber* data from ODP 999A for comparison, from Foster (2008). The results of boron-based $p\text{CO}_2$ reconstruction at these sites are also plotted in Figure 7b, with the estimates from ODP 999A corrected using a pre-industrial $\Delta p\text{CO}_2$ of -21 ppm calculated as discussed above. A cubic spline was plotted using Analyseries (Paillard et al., 1996), and the bounds of uncertainty (17 ppm: individual uncertainty of $29 \text{ ppm} / \sqrt{2}$) on this spline are shaded. For comparison, the data are plotted with atmospheric $p\text{CO}_2$ derived from ice cores (Lüthi et al., 2008 and references within; Laurantou et al., 2010; ages recalculated as per Lemieux-Dudon et al., 2010) and $p\text{CO}_2$ reconstructed assuming a constant vital effect of 0.8 ‰ (as per Foster 2008). The mean deviation from atmospheric $p\text{CO}_2$ measurements from ice cores and those calculated from 999A and GeoB1523-1 using our new calibration is -5 ppm, with a 2σ of ± 19 ppm.

547

548 4 DISCUSSION

549

550 4.1. pH sensitivity of $\delta^{11}\text{B}_{G.ruber}$ lower than $\delta^{11}\text{B}_{borate}$

551 pH is clearly a strong control on $\delta^{11}\text{B}$ in *G. ruber*, yet these new data
552 do not fall on the predicted relationship of seawater borate ion, as epifaunal
553 benthic foraminifera do (albeit within a limited range of $\delta^{11}\text{B}_{borate}$; Rae et al.
554 2011). Specifically, our data lie above the $\delta^{11}\text{B}_{borate}$ -pH curve, and show a pH
555 sensitivity ~40 % lower than that predicted for aqueous $\delta^{11}\text{B}_{borate}$, confirming
556 previous observations in other symbiont-bearing planktonic species (Hönisch
557 et al., 2003; Sanyal et al., 2001, 1996) using NTIMS. This suggests that,
558 absolute values aside, relative changes in $\delta^{11}\text{B}$ are still accurately described
559 using N-TIMS (Foster et al., in review, Chem. Geol.). The causes of this
560 deviation from the aqueous geochemical basis of the proxy are potentially
561 manifold. Previous studies have ascribed a lower-than-predicted pH
562 sensitivity in $\delta^{11}\text{B}$ to: (i) incorporation of $\text{B}(\text{OH})_3$ (Klochko et al., 2009); (ii)
563 elevated (compared to ambient) pH inside seawater vacuoles (Rollion-Bard
564 and Erez, 2010); (iii) modification of the micro-environment around the
565 foraminifera by respiration, calcification and photosynthesis (Hönisch et al.,
566 2003; Zeebe et al., 2003); and (iv) analytical issues relating to the NTIMS
567 approach used in past studies (Foster, 2008). These new results for *G. ruber*
568 using MC-ICPMS, where accuracy has been demonstrated (Ni et al., 2010),
569 show that analytical issues do not play a role in generating the shallower pH
570 sensitivity in planktic foraminifera. Offsets in *absolute* $\delta^{11}\text{B}$ between NTIMS
571 and MC-ICPMS of the order of 1-2 ‰ are observed for a variety of

572 foraminiferal species (e.g., *Cibicidoides weullerstorfi*; see Rae et al., 2011)
573 but, as also supported by an ongoing interlaboratory comparison study
574 (Foster et al., in review, Chem. Geol.), *relative* changes appear to be largely
575 reproducible between NTIMS and MC-ICPMS (e.g. Figure 8 of Rae et al.,
576 2011). There currently appears to be little consensus regarding the relative
577 importance of the other three phenomena proposed above to explain a
578 shallower-than-predicted relationship between $\delta^{11}\text{B}$ and pH. A key
579 observation requiring explanation is that all planktonic foraminiferal
580 calibrations to date show a pH sensitivity (slope 'm') within uncertainty of that
581 seen in inorganic precipitates ($\delta^{11}\text{B}_{\text{CaCO}_3} = \sim 0.75 (\pm 0.15) \times$ the pH sensitivity
582 of $\delta^{11}\text{B}_{\text{borate}}$; Sanyal et al. 2000). Similarly reduced pH sensitivity is evident in
583 the symbiont-bearing *Amphistegina lobifera* (Rollion-Bard and Erez, 2010)
584 and in numerous species of coral (Hönisch et al., 2004; Krief et al., 2010;
585 Trotter et al., 2011; McCulloch et al., 2012). That the sensitivities observed in
586 a broad range of biogenic carbonates are similar to those seen in inorganic
587 precipitates would implicate an inorganic process – e.g., a rate dependency in
588 CaCO_3 precipitation. However, the epifaunal benthic data of Rae et al. (2011;
589 Fig. 2) and Yu et al. (2010) lie within uncertainty of the $\delta^{11}\text{B}_{\text{borate}}$, implying that
590 either the agreement between the $\delta^{11}\text{B}_{\text{borate}}$ and benthic foraminiferal
591 carbonate is either purely fortuitous (the result of a several competing
592 processes operating in tandem to cancel each other out), or that any inorganic
593 process, if it exists, is not universal and benthic foraminifera are the exception
594 to the rule. It is also worth noting that all the other foraminifera so far
595 calibrated are symbiont bearing, and laboratory observations (Jørgensen et
596 al., 1985; Köhler-Rink and Kühl, 2005; Rink et al., 1998) would strongly

implicate the alteration of the foraminiferal microenvironment by symbionts and life processes. However, a full resolution of this issue is beyond the scope of this current contribution and will require additional studies of both symbiont-bearing and non-symbiont bearing foraminifera, and of inorganic carbonates precipitated in equilibrium at rates comparable to those seen in biogenic carbonates.

4.2 $\delta^{11}\text{B}$ offset between culture and non-culture: a size fraction effect on $\delta^{11}\text{B}$ in *G. ruber*

While towed, sediment trap and core-top specimens are permissively in agreement with the pH-sensitivity of $\delta^{11}\text{B}_{\text{CaCO}_3}$ observed in our cultures, it is clear there is a discrepancy in $\delta^{11}\text{B}$ values predicted by the culture calibration and some measurements of non-culture specimens of *G. ruber* (Fig. 5). The degree of offset from the calibration does not correlate with *in situ* temperature ($R^2 = 0.04$) or the saturation state of bottom water at these coretop sites ($R^2 = 0.05$), suggesting there is no discernible temperature or dissolution effect. This offset does, however, show some correlation with test size fraction (see Fig. 6, Supplementary Fig. 8). Previous evidence (Ni et al., 2007) for a size-fraction effect in *G. ruber* is ambiguous because of prohibitive uncertainty in $\delta^{11}\text{B}$ measurements ($\sim \pm 0.8 \text{ ‰}$ vs. $\sim \pm 0.25 \text{ ‰}$ here). Increasing $\delta^{11}\text{B}$ with size is, however, resolvable in the closely-related species *G. sacculifer* (Hemming and Hönisch, 2007; Ni et al., 2007). Moreover, size-fraction effects in *G. ruber* have been noted in $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ (e.g. Kroon and Darling, 1995), B/Ca (Ni et al., 2007), and in Mg/Ca and Sr/Ca (e.g. Friedrich et al., 2012).

The cause of any size fraction effect on $\delta^{11}\text{B}$ is not clear. Preferential dissolution (Hönisch and Hemming, 2004; Ni et al., 2007) seems unlikely, since a) similar offsets from aqueous $\delta^{11}\text{B}_{\text{borate}}$ are reproduced even in towed samples (see Fig. 5), b) there is no relationship between degree of offset and deepwater carbonate saturation at the site of deposition, and c) the lack of any geochemically distinct gametogenic calcite in *G. ruber* (Caron et al., 1990) should make the issue of preferential dissolution less pertinent in this species (see also Seki et al., 2010, Fig. 7a). Instead we support the suggestion by Hönisch and Hemming (2004) that a size-fraction effect in $\delta^{11}\text{B}$ is due to intensified micro-environmental alteration by symbionts in larger specimens. That said, we query the assertion by Hönisch and Hemming (2004) that increasing $\delta^{13}\text{C}$ and $\delta^{11}\text{B}$ with size must be due to larger specimens living at shallower depths (thus experiencing a stronger light intensity and stronger microenvironment alteration). While this seems consistent with Caron et al. (1982), we urge some caution in this interpretation without direct ecological evidence for a bias towards larger test size in *G. ruber* from shallower waters: Bijma et al. (1990) report living individuals measuring anywhere between 125 μm and 708 μm within the uppermost 5 m of the Red Sea at all stages in *G. ruber*'s semilunar life-cycle. Besides, foraminifera towed from < 10 m record lower, not higher, $\delta^{11}\text{B}$ than foraminifera cultured at light levels equivalent to ~20 m water depth (though we concede these individuals might conceivably have grown to larger size – and heavier $\delta^{11}\text{B}$ – had they completed their life cycle at this depth).

It is instead likely that the magnitude of these microenvironment effects

might change with test size without any need to invoke changes in habitat depth. Photosynthesis by symbionts in the microenvironment surrounding planktic foraminifera raises pH, while respiration and calcification lower pH (Jørgensen et al., 1985; Köhler-Rink and Kühl, 2005; Rink et al., 1998). Crucially, rates of respiration and photosynthesis in culture specimens have been seen to change with test size (Lombard et al., 2009; Rink et al., 1998), with photosynthesis increasing relative to respiration in larger specimens of *G. ruber* (Lombard et al., 2009). In addition, as foraminifera grow, the diffusive boundary layer around their tests is expanded, lengthening timescales for diffusion of carbon through the microenvironment. As such, equilibration of the microenvironment with the ambient seawater slows, and as such any microenvironment pH alteration would be accentuated (see model of Wolf-Gladrow et al., 1999). While more *in situ* microelectrode measurements are required to fully test these hypotheses, they could explain the observed patterns in recorded $\delta^{11}\text{B}$ we see here.

It is also possible that increased test size might be resultant from, not a driver of, increased symbiont activity, with elevated $\delta^{11}\text{B}$ an inevitable result. Planktonic foraminifera acquire algal symbionts early in their life cycle (Hemleben et al., 1989). Should the activity of symbionts in the foraminiferal microenvironment impart an advantage to the calcification of the host by raising external pH and Ω_{CaCO_3} (reflected in $\delta^{11}\text{B}$) and thus reducing energetic expenditure needed to raise internal vacuole pH (as suggested by Bentov et al., 2009), foraminifera that happen to succeed in acquiring symbionts earlier in their ontogeny might grow more rapidly, and thus attain larger size prior to

gametogenesis. Clearly additional modelling and field observation are required to investigate these hypotheses further.

*4.3 Applying the *G. ruber* $\delta^{11}\text{B}$ -pH calibration to downcore data*

Reconstructed pCO_2 from Sites GeoB1523-1 and ODP 999A using this new calibration for *G. ruber* tracks reconstructions from ice cores very closely, with average deviation (~ 5 ppm) from ice core pCO_2 within propagated uncertainty of ± 29 ppm. Although broadly similar results can be generated with the approach of Foster (2008; equation 6 above), the new calibration more accurately reproduces the 90 ppm magnitude of deglacial pCO_2 increase seen in ice core reconstructions ($\Delta\text{pCO}_2 = \sim 80$ ppm vs. ~ 50 ppm using Foster 2008). What is more, while these sorts of improvements in fit are within uncertainty over these short timescales, the importance of the lower pH sensitivity documented here is magnified in deeper time when pCO_2 is likely to have been higher.

5 Conclusions

This contribution represents a further demonstration of the dominance of pH as a control on foraminiferal $\delta^{11}\text{B}$. It is the first species-specific foraminiferal culture calibration analysed using MC-ICPMS, and the first to incorporate globally-distributed cultured, towed, sediment trap and core-top foraminifera. The new method we advocate for presenting culture calibrations allows for greater ease of comparison and uncertainty calculation and

propagation. We show that recorded $\delta^{11}\text{B}$ in *G. ruber* deviates markedly from the simple inorganic basis of the proxy, corroborating previous foraminiferal culture studies analysed using N-TIMS (Sanyal et al., 2001, 1996), and supporting the results of published down-core reconstructions that are based on pH-sensitivities of $\delta^{11}\text{B}_{\text{CaCO}_3}$ that are lower than $\delta^{11}\text{B}_{\text{borate}}$. We also document for the first time a size-fraction effect in $\delta^{11}\text{B}_{G. ruber}$, which must be taken into account in down-core application. As we illustrate for the last 30 kyr (Fig. 7), however, by analysing well-constrained size fractions and applying a species-specific culture calibration, accurate and precise estimates of past levels of atmospheric CO_2 can be reconstructed.

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FIGURE CAPTIONS

Figure 1: (a) The relative distribution of boron species in seawater over a range of pH at 25 °C and 35 psu, with pK_B^* calculated according to Dickson (1990). (b) The changing isotopic composition of each species according to equation (3) in the text.

Figure 2: Published planktic foraminiferal culture and inorganic precipitate experiments, plotted in $\delta^{11}\text{B}$ -pH space (top panel) and in $\delta^{11}\text{B}_{\text{CaCO}_3}$ - $\delta^{11}\text{B}_{\text{borate}}$ space (bottom panel). Note that for the purposes of graphical representation, the data were normalised to a $i^{11}\text{B}_{\text{sw}} = 39.61 \text{ ‰}$ (both panels), and to a temperature of 26°C and a salinity of 37.2 psu (the conditions of our culture calibration, top panel only). The black line in ^{11}B -pH space is the aqueous value of $\delta^{11}\text{B}_{\text{borate}}$ at these environmental conditions, with the dotted lines representing the uncertainty on the value of $^{11-10}K_B$ (0.0006) in seawater at 25 °C reported by Klochko et al. (2006). Coloured calibration lines are best fits, varying $^{11-10}K_B$ and a from Equation (6). The black line in $\delta^{11}\text{B}_{\text{CaCO}_3}$ - $\delta^{11}\text{B}_{\text{borate}}$ space (bottom panel) is a 1:1 relationship, i.e. a pH sensitivity equal to that of borate ion. Calibrations are represented by York regressions calculated using Isoplot (Ludwig 2003), with shaded areas representing 95% confidence intervals for these regressions. Note that it was not possible to calculate 95% confidence intervals for the data of Rae et al. (2011) or Foster (2008) using this method, as the spread in $\delta^{11}\text{B}_{\text{borate}}$ is insufficient, but they are included for comparison, having been used elsewhere in downcore reconstructions. Carbonate system parameters for Sanyal et al. (1996, 2000, 2001) are adjusted from NBS scale to total scale by subtraction of -0.14. While this is no doubt more representative than no correction at all, we emphasise that NBS-total scale conversions are not necessarily universal and that the correction

used is critical to the resultant slope (see Supplementary Fig. 1). As such we emphasise that the Sanyal calibrations are plotted more for comparison of trend than for comparison of exact positioning of that trend on any one axis.

Figure 3: Locations of core-top, tow and sediment trap samples used in this study. Filled circles are sites from which recent samples are measured (this study), while white circles are core-tops from Foster (2008). Sites of down-core pCO₂ reconstructions are marked by a grey square (GeoB1523-1) and white circle (ODP 999A: also the site of core-top measurement; Foster 2008).

Figure 4: Examples of foraminifera grown in culture: (A) healthy *G. ruber* at ambient pH, with symbionts spread out within a well-developed halo of spines; (B) Foraminifera grown under low pH (~7.55), with poorly developed spines and dense shroud of symbionts; (C) Test grown at high pH (~8.17) showing erratic pattern of chamber addition, also seen (albeit less frequently) at mid-range pH (D) 'Normal' test of *G. ruber*, similar to those seen in sediment samples (e.g. Hemleben et al., 1989), as produced in all three pH experiments; (E) Test with large apertures and thinly calcified chambers that may be indicative of dissolution, as sometimes seen at low pH (~7.55). Note that examples C and E are not representative of the whole experimental group, but are extremes picked for illustration purposes.

Figure 5. New culture calibration of *G. ruber*. York-fit regression plotted using Isoplot (Ludwig 2003), with dotted lines and grey band defining 95% confidence intervals (mean standard weighted deviance (MSWD) = 0.01). X-error bars for core-top samples are 2 standard deviations of intra-annual variability in calculated monthly $\delta^{11}\text{B}_{\text{borate}}$, while for cultures the error represents 2 standard errors of the mean pH of all culture flasks, and for sediment trap samples it reflects the range of $\delta^{11}\text{B}_{\text{borate}}$ between Dec-Feb 2007. Y-error in non-culture samples is the analytical reproducibility as calculated by equation 10. In cultured samples, error bars reflect 2 standard deviations of 10,000 Monte Carlo simulations that also incorporate the uncertainties in the size-mass relationship (see Supp. Figure 4b) and reproducibility of boron isotope and B/Ca measurements in 'control' pre-culture tows and bulk post-culture measurements.

Figure 6. Offset of core-top, tow and sediment trap samples from our culture calibration, as a function of size fraction. Error bars in size-fraction refer to the sieve fractions, or where available, two standard errors of the measured test diameter. Red markers indicate the mean value for the three most commonly used size fractions, with y-errors corresponding to two standard errors of the mean offset. Dotted vertical line denotes the mean end-culture diameter.

Fig. 7. Down-core $\delta^{11}\text{B}$ (Panel A) and reconstructed pCO₂ (Panel B) from ODP site 999A (crosses, Foster, 2008) and GeoB1532-1 (circles, this study), compared to CO₂ concentrations from ice cores (black line). The thick red line in Panel B is a cubic spline plotted on evenly subsampled values from both sites, as calculated using Analyseries (Paillard et al., 1996), with the shaded area reflecting uncertainty on the spline of ± 17 ppm (i.e. 29 ppm individual uncertainty / $\sqrt{2}$). Note that these data are corrected for local ocean-

atmosphere disequilibrium, which is taken as the modern mean annual values (from Takahashi et al. 2009) corrected for the pre-industrial with reference to Gloor et al. (2003): +21 ppm (999A) and +27 ppm (GeoB1523-1). Also plotted (green line) is a similar spline through pCO₂ reconstructions based on a constant vital effect 'a' (see equation (6) above) of +0.8 ‰ as applied by Foster (2008), illustrating the improved fit of the new calibration. Uncertainty on each individual measurement is plotted (Panel A), with uncertainty on pCO₂ reconstructions (Panel B) a quadratic addition of the various uncertainties on reconstructed pH, alkalinity and temperature. Insert in panel B is a cross-plot of ice-core derived vs. reconstructed pCO₂, with uncertainty on the 1:1 line (shown as grey shaded region) representing 6 ppm uncertainty on ice-core measurements (from Ahn et al., 2012).

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1114

- We present a new $\delta^{11}\text{B}$ -pH calibration for the planktonic foraminifera *G. ruber*
- We see a lower pH sensitivity in the $\delta^{11}\text{B}$ of *G. ruber* compared to $\delta^{11}\text{B}_{\text{borate}}$.
- We find a small but consequential effect of test size on *G. ruber* $\delta^{11}\text{B}$.
- We discuss the significance in terms of deciphering foraminiferal 'vital effects'
- Calibration applied to the last 30 kyr accurately reproduces ice core values.

Fig3.pdf

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Fig2.pdf
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$$\delta^{11}B_{\text{borate}} = \left(\frac{\delta^{11}B_{\text{measured}} - c}{m} \right)$$

Fig6.pdf

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Publication	Carbonate type	Coefficients of calibration				MSWD ^d	<i>p</i> ^e
		$\delta^{11}B_{\text{borate}} = (\delta^{11}B_{\text{CaCO}_3} - c) / m$					
		<i>c</i>	<i>2σ</i>	<i>m</i>	<i>2σ</i>		
Sanyal <i>et al.</i> (1996) ^{abc}	<i>Orbulina universa</i>	5	5.3	0.82	0.32	5.6	0.004
Sanyal <i>et al.</i> (2000) ^a	Inorganic precipitates	7.1	3	0.75	0.15	0.118	0.73
Sanyal <i>et al.</i> (2001) ^{ab}	<i>Globigerinoides sacculifer</i>	6.7	3.3	0.85	0.19	1.05	0.3
this study	<i>Globigerinoides ruber</i>	9.52	1.51	0.6	0.08	0.01	0.96

^a pH measurements (from which $\delta^{11}B_{\text{borate}}$ is derived) come from NBS-buffer-calibrated electrode measurements, and are approximated to total scale by subtraction of 0.14.
^b Salinity assumed to be 35 psu
^c 'Room temperature' assumed to be 20 °C
^d MSWD = Mean Square Weighted Deviation
^e *p* = probability of fit at 95% confidence
York-fit regression statistics from Isoplot (Ludwig 2003).

Experiment	Start/Tow date	pH (total)	2se	T (°C)	S	N (cultured) ^a	N (grown and GAM) ^b	Survivorship (%) ^c	Mean days in culture	Mean starting diameter (µm) ^d	Mean end-culture diameter (µm) ^d	Mass for analysis (µg) ^e	Growth rate (µg/day) /ind ^f
Culture 1	29/01/2010	8.174	0.007	26	37	106	57	54	6	200.0	365.4	590	1.39
Culture 2	10/02/2010	7.894	0.013	26	37	105	67	64	10	178.1	441.9	902	1.19
Culture 3	10/03/2010	7.554	0.013	26	37	120	48	40	9	197.7	339.6	303	0.35
Tow 1	27/01/2010	8.128	0.005	22	40								
Tow 2	08/02/2010	8.116	0.005	23	40								
Tow 3	07/03/2010	8.103	0.005	23	40								

^a Indicates the number of foraminifera grown at each pH treatment
^b Indicates the number of foraminifera that grew in mass during culture and underwent gametogenesis
^c The percentage of individuals cultured that grew and survived to gametogenesis in culture.
^d As determined by micrometer and inverted light microscope
^e The total mass of calcite analysed via MC-ICPMS, of which 75-92 % was grown in culture

^f Based on the pre-culture mass as estimated from the size-mass relationship in Supplementary Figure 2 and the end-culture mass as weighed at the University of Bristol, and represents the mean of the growth rates of each individual foraminifera per treatment

Sample	type	Core/Trap/Tow		Latitude (°)	Longitude (°)	T (°C) ^b	S ^c	pK ^{a, d} _s	total	pCO ₂	pH	2σ ^h	δ ¹¹ B _{borate} ⁱ	2σ ^l	δ ¹¹ B _{calcite} ^k	2σ ^j	P ^a _{culture} ^m	δ ¹¹ B _{corrected} ⁿ	2σ ^o
		Depth (m)	Age (kyr BP) ^a						Alkalinity										
									(μmol/kg) ^e	(μatm) ^f	(total) ^g								
Eilat, Culture 1	Culture	n/a	n/a	29.50	34.92	26	37.2	8.574	2392	277	8.184	0.007	19.76	0.09	21.35	0.23	0.907	21.46	0.26
Eilat, Culture 2	Culture	n/a	n/a	29.50	34.92	26	37.2	8.574	2189	576	7.904	0.013	16.66	0.12	19.66	0.21	0.945	19.63	0.25
Eilat, Culture 3	Culture	n/a	n/a	29.50	34.92	26	37.2	8.574	2027	1415	7.564	0.013	14.22	0.06	18.72	0.31	0.770	18.20	0.43
Eilat, Tow 1	Tow	< 10	n/a	29.50	34.92	22	40.40	8.605	2500	330	8.128	0.005	18.83	0.06	20.25	0.22			
Eilat, Tow 2	Tow	< 10	n/a	29.50	34.92	23	40.32	8.594	2508	342	8.116	0.005	18.82	0.06	20.23	0.33			
Eilat, Tow 3	Tow	< 10	n/a	29.50	34.92	23	40.44	8.593	2500	354	8.103	0.005	18.68	0.06	20.44	0.23			
CAR-22(z), <i>sensu stricto</i> , 250-300 μm	Sediment trap	150	n/a	10.50	64.66	24.17	36.70	8.598	2412	388	8.066	0.018	18.21	0.10	20.05	0.19			
CAR-22(z), <i>sensu stricto</i> , 300-355 μm	Sediment trap	150	n/a	10.50	64.66	24.17	36.70	8.598	2412	388	8.066	0.018	18.21	0.10	20.17	0.19			
Eilat, <i>sensu stricto</i> , 250-300 μm	Grab sample	300	Holocene	29.50	34.92	23.76	40.72	8.582	2510	275	8.188	0.008	19.84	0.28	20.31	0.21			
Eilat, <i>sensu stricto</i> , 300-355 μm	Grab sample	300	Holocene	29.50	34.92	23.76	40.72	8.582	2510	275	8.188	0.008	19.84	0.28	21.25	0.42			
MC 497, <i>sensu stricto</i> , 300-355 μm	Core-top	1000	Holocene	23.53	63.31	26.86	36.37	8.568	2376	295	8.152	0.020	19.57	0.45	20.87	0.18			
MC 497, <i>sensu stricto</i> , 355-400 μm	Core-top	1000	Holocene	23.53	63.31	26.86	36.37	8.568	2376	295	8.152	0.020	19.57	0.45	20.97	0.17			
MC 497, <i>sensu stricto</i> , 400-455 μm	Core-top	1000	Holocene	23.53	63.31	26.86	36.37	8.568	2376	295	8.152	0.020	19.57	0.45	21.87	0.18			
GGC-48, <i>mixed morphotype</i> , 250-300 μm	Core-top	3055	Holocene	0.00	161.00	29.41	34.61	8.549	2276	329	8.102	0.066	19.20	0.81	20.14	0.20			
GGC-48, <i>mixed morphotype</i> , 300-355 μm	Core-top	3055	Holocene	0.00	161.00	29.41	34.61	8.549	2276	329	8.102	0.066	19.20	0.81	20.21	0.33			
ODP 664, <i>sensu stricto</i> , 300-355 μm	Core-top	3817	2.9-3.6	0.10	-23.23	26.81	35.77	8.572	2341	306	8.137	0.073	19.26	0.71	20.82	0.19			
G4, <i>sensu stricto</i> , 300-355 μm	Core-top	831	0.9	-28.42	167.25	21.95	35.82	8.628	2361	260	8.202	0.030	19.44	0.29	20.64	0.19			
G4, <i>sensu stricto</i> , 355-400 μm	Core-top	831	0.9	-28.42	167.25	21.95	35.82	8.628	2361	260	8.202	0.030	19.44	0.29	21.43	0.20			
T329, <i>sensu stricto</i> , 250-300 μm	Core-top	451	2.9	-12.96	173.57	28.63	34.79	8.556	2288	270	8.171	0.024	19.96	0.27	20.83	0.18			
T329, <i>sensu stricto</i> , 300-355 μm	Core-top	451	2.9	-12.96	173.57	28.63	34.79	8.556	2288	270	8.171	0.024	19.96	0.27	20.73	0.19			
T329, <i>sensu stricto</i> , 355-400 μm	Core-top	451	2.9	-12.96	173.57	28.63	34.79	8.556	2288	270	8.171	0.024	19.96	0.27	21.29	0.16			
OC476-SR223, <i>sensu stricto</i> , 250-355 μm	Core-top	2860	0.7	-33.53	166.53	19.33	35.70	8.660	2353	262	8.201	0.013	19.04	0.37	20.24	0.38			
999A	Downcore	2827+0.05	3.9	12.75	-78.73	28.23	35.5	8.557	2330	282	8.15	0.03			20.67	0.25		19.63	0.35
999A	Downcore	2827+0.11	4.6	12.75	-78.73	28.44	35	8.557	2301	265	8.16	0.03			20.78	0.25		19.82	0.37
999A	Downcore	2827+0.15	5	12.75	-78.73	27.77	35	8.561	2301	283	8.14	0.03			20.59	0.25		19.51	0.34
999A	Downcore	2827+0.31	7.8	12.75	-78.73	28.19	35.55	8.557	2333	264	8.17	0.03			20.82	0.25		19.89	0.38
999A	Downcore	2827+0.31	7.8 (2nd Rep)	12.75	-78.73	28.19	35.55	8.557	2333	276	8.15	0.03			20.72	0.25		19.71	0.36
999A	Downcore	2827+0.39	9.5	12.75	-78.73	27.59	36.1	8.562	2366	279	8.15	0.03			20.68	0.25		19.65	0.36
999A	Downcore	2827+0.43	10.3	12.75	-78.73	27.50	36.4	8.561	2384	254	8.18	0.03			20.93	0.25		20.06	0.40
999A	Downcore	2827+0.48	11.7	12.75	-78.73	27.04	36.51	8.567	2390	272	8.16	0.03			20.72	0.25		19.72	0.36
999A	Downcore	2827+0.57	14	12.75	-78.73	27.38	36.82	8.560	2408	251	8.19	0.03			20.98	0.25		20.15	0.41
999A	Downcore	2827+0.61	15.1	12.75	-78.73	27.41	37.1	8.559	2425	249	8.19	0.03			21.02	0.25		20.22	0.42
999A	Downcore	2827+0.61	15.1 (2nd Rep)	12.75	-78.73	27.41	37.1	8.559	2425	237	8.21	0.03			21.15	0.25		20.43	0.44
999A	Downcore	2827+0.71	17.3	12.75	-78.73	26.69	37.11	8.569	2426	240	8.21	0.03			21.04	0.25		20.25	0.42
999A	Downcore	2827+0.82	19.2	12.75	-78.73	26.17	37	8.575	2419	176	8.30	0.04			21.75	0.25		21.43	0.57
999A	Downcore	2827+0.93	21.1	12.75	-78.73	26.01	36.95	8.579	2416	219	8.24	0.03			21.20	0.25		20.51	0.45
999A	Downcore	2827+1.02	22.5	12.75	-78.73	26.69	37.18	8.569	2430	220	8.23	0.03			21.26	0.25		20.62	0.47
999A	Downcore	2827+1.13	24.8	12.75	-78.73	26.58	37.05	8.573	2422	195	8.27	0.04			21.51	0.25		21.04	0.52
999A	Downcore	2827+1.20	26.3	12.75	-78.73	26.34	36.84	8.575	2410	185	8.28	0.04			21.62	0.25		21.22	0.54
999A	Downcore	2827+1.20	26.3 (2nd Rep)	12.75	-78.73	26.34	36.84	8.575	2410	180	8.29	0.04			21.69	0.25		21.33	0.56
GeoB1523-1	Downcore	3292+0.05	3.1	3.83	-41.62	27.76	35.54	8.563	2333	259	8.17	0.03			20.78	0.24		19.81	0.38
GeoB1523-1	Downcore	3292+0.10	6	3.83	-41.62	27.40	35.64	8.566	2338	264	8.16	0.02			20.72	0.23		19.71	0.36
GeoB1523-1	Downcore	3292+0.15	8.5	3.83	-41.62	27.62	35.86	8.562	2352	240	8.19	0.03			20.97	0.24		20.14	0.42
GeoB1523-1	Downcore	3292+0.20	10.5	3.83	-41.62	26.94	36.10	8.569	2366	262	8.17	0.03			20.73	0.24		19.74	0.37
GeoB1523-1	Downcore	3292+0.25	12.6	3.83	-41.62	26.52	36.33	8.572	2379	206	8.24	0.03			21.29	0.23		20.66	0.48
GeoB1523-1	Downcore	3292+0.30	14.6	3.83	-41.62	26.22	36.52	8.575	2391	220	8.22	0.03			21.13	0.24		20.39	0.45
GeoB1523-1	Downcore	3292+0.35	16.6	3.83	-41.62	24.83	36.64	8.590	2398	194	8.26	0.04			21.33	0.26		20.73	0.50
GeoB1523-1	Downcore	3292+0.40	18.2	3.83	-41.62	25.26	36.69	8.585	2401	194	8.26	0.04			21.37	0.26		20.79	0.51
GeoB1523-1	Downcore	3292+0.45	19.1	3.83	-41.62	24.85	36.69	8.590	2401	209	8.24	0.03			21.15	0.25		20.43	0.46
GeoB1523-1	Downcore	3292+0.55	20.9	3.83	-41.62	25.76	36.65	8.580	2399	217	8.23	0.03			21.14	0.26		20.42	0.46
GeoB1523-1	Downcore	3292+0.65	23.9	3.83	-41.62	25.22	36.51	8.587	2390	225	8.22	0.03			20.99	0.25		20.17	0.43
GeoB1523-1	Downcore	3292+0.75	27.4	3.83	-41.62	25.31	36.34	8.587	2380	208	8.24	0.03			21.17	0.25		20.47	0.47
GeoB1523-1	Downcore	3292+0.85	32.9	3.83	-41.62	24.88	36.28	8.592	2376	253	8.18	0.02			20.67	0.25		19.64	0.37

^a Core-top sediments were where possible determined as undisturbed Holocene by either the presence of stained benthic foraminifera (MC 497), existing age-model (ODP 664, Raymo et al. 1997; GGC48, E. Tappa pers. comm.) or carbon dating (G4, T329, OC476-SR223; Bostock, pers. comm.). Down-core ages were derived from existing age models by Schmidt et al. (2004; 999A) and Gingele et al. (2000; GeoB1523-1).

^b For tow and culture samples, temperature was gauged by direct measurement. For sediment trap samples, temperature interpolated from data from www.imars.usf.edu/CAR/ (see Supplementary Information). Core-top temperature is mean annual sea surface temperature interpolated from nearby sites from the Takahashi et al. (2009) database. Downcore temperatures for 999A are interpolated from Schmidt et al. (2004), and at GeoB1523-1 are derived from Mg/Ca data (see Supplementary Information) and the general calibration of Anand et al. (2003).

^c For tow and culture samples, salinity was gauged by direct measurement. For sediment trap samples, salinity interpolated from data from www.imars.usf.edu/CAR/ (see Supplementary Information). Core-top salinity is mean annual salinity interpolated from nearby sites from the Takahashi et al. (2009) database. Downcore salinity for 999A and GeoB1523-1 are derived from sea level estimates.

^d Calculated from temperature, salinity and depth.

^e For tow and culture samples, alkalinity was gauged by direct measurement. For sediment trap samples, alkalinity interpolated from data from www.imars.usf.edu/CAR/ (see Supplementary Information). Core-top alkalinity is derived from regional salinity-alkalinity relationships (from GLODAP/CARINA data <20m deep) and mean annual salinity interpolated from nearby sites from the Takahashi et al. (2009) database. Downcore alkalinity for 999A is from Foster (2008) and for GeoB1523-1 is derived from reconstructed salinity.

^f For tows, culture and downcore samples, pCO₂ was reconstructed from alkalinity and either pH reconstructed using the new calibration (downcore) or Total DIC as measured directly (tows, cultures). For sediment trap samples, pCO₂ is interpolated from data from www.imars.usf.edu/CAR/ (see Supplementary Information). Core-top pCO₂ is reconstructed by application of the mean annual ΔpCO₂ at each site (interpolated from nearby sites from Takahashi et al. 2009 and corrected for post-industrial changes in ΔpCO₂ according to model results from Gloor et al. 2003) to pre-industrial atmospheric CO₂ concentrations.

^g For tows and sediment traps, pH is calculated from measurements of alkalinity and DIC (tows) or pCO₂ (sediment traps), while for core-tops the pH is a mean annual pH interpolated from nearby sites from Takahashi et al. (2009) and calculated from estimated alkalinity and pCO₂. For cultured samples, pH is calculated as the mean of the mean pH observed by each individual foraminifera during culture (as measured using a potentiometric electrode but cross-calibrated to DIC/TALK-derived pH). For down-core samples, pH is derived

from δ¹¹B measurements corrected using the new calibration for *G. ruber*.

^h Uncertainty on culture pH measurements is quoted as 2 standard errors of the mean of the average pHs experienced by constituent forams, while uncertainty on the pH estimates for tows is based on analytical reproducibility at the UK-OARP Carbonate Chemistry Facility. Uncertainty on core-top pH is the interpolated intra-annual variability in pH at that site (see Supplementary Materials), while in down-core samples it signifies the pH uncertainty resultant from the uncertainty on corrected δ¹¹B_{CaCO₃}. At Cariaco basin (*), pH is interpolated between cruise measurements taken a month either side of sediment trap deployment, and uncertainty reflects the range in pH between these two months.

ⁱ As calculated from given pH and pK_b using the general boron isotope-pH equation (5)

^j Reflecting the uncertainty in pH and pK_b

^k Includes published data from 999A from Foster (2008) that are re-interpreted here. Measured via ICPMS at Bristol (cultures, tows and downcore) and Southampton (core-tops and sediment traps)

^l Uncertainty on δ¹¹B measurements is derived from the long-term external reproducibility of standards at Bristol (Cultures, Tows, GeoB1523-1), and NOCS (core-tops, sediment traps), except in case of the published data from 999A (Foster 2008) where the published uncertainty is quoted.

^m Proportion of Boron incorporated during culture, as calculated via mass balance of measured B/Ca; see Equation (8)

ⁿ δ¹¹B_{corrected} for culture samples is the δ¹¹B of carbonate grown under culture conditions, having been mass-balance corrected for test mass grown out of culture. For downcore samples, it refers to δ¹¹B derived from transformation of raw δ¹¹B data using the new *G. ruber* calibration presented here (analogous to δ¹¹B_{borate*} and used to reconstruct palaeo-pH).

^o Uncertainty on δ¹¹B_{corrected} is 2 standard deviations of 10,000 Monte Carlo simulations incorporating reproducibility in δ¹¹B and B/Ca measurements of towed 'control' and culture foraminifera, and the uncertainty in size-weight calibration (for culture samples), or a quadratic addition of measurement reproducibility and calibration uncertainty (down-core samples).

All carbonate system and pK_b calculations use CO2sys.m (van Heuven et al., 2011) and the constants of Dickson (1990), Lueker et al. (2000) and Lee et al. (2010). Further details may be found in the Supplementary Information. Note that we see no influence of boron from contaminant clays, with Al/Ca ratios all below 100 μmol/mol.

Supplementary Figure 1: Published bulk planktic foraminiferal culture and inorganic precipitate experiments, as in Fig. 2b, but plotted with a larger NBS-total scale correction as we observed here (NBS - 0.21) in panel A, and without any correction to total scale in panel B. Note the difference in slopes and thus the importance good carbonate system characterization for these sorts of calibrations. Given that NBS-total scale conversions are not universal (Wedborg et al. 1999), these data must be viewed with caution. Note that if the Sanyal et al. data were corrected by the same NBS-total offset as this study, their slopes would be within uncertainty of the pH sensitivity of aqueous borate of Klochko et al. (2006).

Supplementary Figure 2: Measurements of *G. ruber* sensu stricto and sensu lato from the same core-top sediment samples, showing no consistent offset. Error bars are calculated according to equation 11.

Supplementary Figure 3: Illustration of the good fit between pH measurements on the total scale calculated from DIC/Alk [using the constants of Lueker et al. (2000) and Dickson (1990)] and NBS-buffer-calibrated electrode measurements once a correction of -0.21 has been applied to the latter. On the basis of this, we derived our culture pH uncertainty from NBS electrode measurements taken regularly during culture, corrected to approximate the total scale.

Supplementary Figure 4: Size:Mass Relationship constructed from towed samples collected between January and March 2010. Masses were determined by weighing individually, while size is defined as the product of the maximum axis (when viewed from the umbilical side) and the axis perpendicular to it. Measurements were taken with Macnification[®] imaging software. Note that this relationship closely resembles that defined by Kısakürek et al. (2011). In panel B, in the log scale, 95% confidence interval on this relationship is plotted using Isoplot (Ludwig 2003).

Supplementary Figure 5: Boron isotope measurements from ODP999A from Foster (2008) cleaned using NaOCl (white squares), compared to samples repicked and cleaned as described in Rae et al. (2011) and measured at NOCS (red squares). The oxygen isotope data from the site are plotted for context.

Supplementary Figure 6: Comparability of measurements of a range of in house standards between the Thermo Neptune MC-ICPMS machines at the Bristol Isotope Group and the National Oceanography Centre, Southampton. Error margins are the quadratic addition of 2 standard deviations on the mean measurements at Bristol and at Southampton.

Supplementary Figure 7: Plot of external reproducibility of JCP (Japanese Coral Porites) standard as a function of ^{11}B intensity (in volts with 10^{11} ohm resistors) measured by MC-ICPMS, following Rae et al. (2011). The line-of-best-fit plotted is a double exponential, described by the relationship

$2\sigma = 1.87 * \exp^{-20.6[^{11}\text{B}]} + 0.22 * \exp^{-0.43[^{11}\text{B}]}$. External reproducibility may then be approximated from observed ^{11}B intensity data, which for the most part is dictated by sample size.

Supplementary Figure 8: Breakdown of the observed size effect in *G. ruber* across different sample sites, highlighting the broadly similar trends seen in all core-top sites. The red line is the expected $\delta^{11}\text{B}$, with the offset from ambient $\delta^{11}\text{B}_{\text{borate}}$ predicted by the average size effect from all sites and size fractions (Fig. 6), while the green line is the mean intra-annual ambient $\delta^{11}\text{B}_{\text{borate}}$ at each site. Uncertainty is calculated as per Equation 10.