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► **To cite this version:**

Ella L. Howes, Karina Kaczmarek, Markus Raitzsch, Antje Mewes, Nienke Bijma, et al.. Decoupled carbonate chemistry controls on the incorporation of boron into *Orbulina universa*. *Biogeosciences*, 2017, 14 (2), pp.415-430. 10.5194/bg-14-415-2017 . hal-01501470

HAL Id: hal-01501470

<https://hal.sorbonne-universite.fr/hal-01501470>

Submitted on 4 Apr 2017

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Decoupled carbonate chemistry controls on the incorporation of boron into *Orbulina universa*

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Received: 14 March 2016 – Published in Biogeosciences Discuss.: 12 May 2016

Revised: 11 November 2016 – Accepted: 20 November 2016 – Published: 26 January 2017

Abstract. In order to fully constrain paleo-carbonate systems, proxies for two out of seven parameters, plus temperature and salinity, are required. The boron isotopic composition ($\delta^{11}\text{B}$) of planktonic foraminifera shells is a powerful tool for reconstructing changes in past surface ocean pH. As $\text{B}(\text{OH})_4^-$ is substituted into the biogenic calcite lattice in place of CO_3^{2-} , and both borate and carbonate ions are more abundant at higher pH, it was suggested early on that B/Ca ratios in biogenic calcite may serve as a proxy for $[\text{CO}_3^{2-}]$. Although several recent studies have shown that a direct connection of B/Ca to carbonate system parameters may be masked by other environmental factors in the field, there is ample evidence for a mechanistic relationship between B/Ca and carbonate system parameters. Here, we focus on investigating the primary relationship to develop a mechanistic understanding of boron uptake.

Differentiating between the effects of pH and $[\text{CO}_3^{2-}]$ is problematic, as they co-vary closely in natural systems, so the major control on boron incorporation remains unclear. To deconvolve the effects of pH and $[\text{CO}_3^{2-}]$ and to investigate their impact on the B/Ca ratio and $\delta^{11}\text{B}$, we conducted culture experiments with the planktonic foraminifer *Orbulina universa* in manipulated culture media: constant pH (8.05), but changing $[\text{CO}_3^{2-}]$ (238, 286 and $534 \mu\text{mol kg}^{-1} \text{CO}_3^{2-}$)

and at constant $[\text{CO}_3^{2-}]$ ($276 \pm 19.5 \mu\text{mol kg}^{-1}$) and varying pH (7.7, 7.9 and 8.05). Measurements of the isotopic composition of boron and the B/Ca ratio were performed simultaneously using a femtosecond laser ablation system coupled to a MC-ICP-MS (multiple-collector inductively coupled plasma mass spectrometer). Our results show that, as expected, $\delta^{11}\text{B}$ is controlled by pH but it is also modulated by $[\text{CO}_3^{2-}]$. On the other hand, the B/Ca ratio is driven by $[\text{HCO}_3^-]$, independently of pH. This suggests that B/Ca ratios in foraminiferal calcite can possibly be used as a second, independent, proxy for complete paleo-carbonate system reconstructions. This is discussed in light of recent literature demonstrating that the primary relationship between B/Ca and $[\text{HCO}_3^-]$ can be obscured by other environmental parameters.

1 Introduction

Before the Anthropocene, the atmospheric CO_2 concentration was governed by the surface ocean $[\text{CO}_2]$, simply because the carbon content of the ocean is 65 times larger than that of the atmosphere (Siegenthaler and Sarmiento, 1993). Hence, understanding the global carbon cycle and the evolu-

tion of atmospheric $p\text{CO}_2$ in Earth history requires knowledge of the dynamics of the oceanic carbonate chemistry. Since the industrial revolution, the unprecedented magnitude and rate of carbon emissions has caused both warming and acidification of the oceans (Bijma et al., 2013; Ciais et al., 2013; Gattuso and Hansson, 2011; Gattuso et al., 2015; Rhein et al., 2013). As a consequence, the interest in the reconstruction of seawater carbonate chemistry to identify ocean acidification in Earth history experienced an impetus (Hönisch et al., 2012; Martínez-Botí et al., 2015a).

The most promising tool for reconstructing pH is the boron isotopic composition ($\delta^{11}\text{B}$) of biogenic carbonate producers such as foraminifera and corals (Hönisch et al., 2004; Rae et al., 2011; Sanyal et al., 2001, 1996; Spivack et al., 1993) and a growing number of studies have thus used $\delta^{11}\text{B}$ -based pH records to reconstruct past atmospheric CO_2 (e.g. Foster et al., 2012, 2006; Hemming et al., 1998; Hönisch et al., 2011, 2008, 2009, 2007, 2012; Hönisch and Hemming, 2005; Martínez-Botí et al., 2015a, b; Palmer et al., 1998; Pearson et al., 2009; Pearson and Palmer, 2000, 1999; Rae et al., 2014; Sanyal and Bijma, 1999; Sanyal et al., 1997, 1995; Seki et al., 2010).

Reconstruction of the full oceanic carbonate chemistry requires proxies of at least two independent parameters of the carbonate system, in addition to temperature and salinity. However, to date, all reconstructions are based on the analysis of $\delta^{11}\text{B}$ of biogenic carbonates alone with assumptions regarding a secondary parameter. In these reconstructions, total alkalinity (A_T) or $[\text{CO}_3^{2-}]$ was estimated from modern ocean conditions or from reconstructions of the carbonate compensation depth (CCD). Total alkalinity is a conservative parameter, meaning that A_T is linearly correlated with salinity (Dickson, 1981, 1992; Wolf-Gladrow et al., 1999, 2007). Therefore, if it is assumed that the modern salinity– A_T relationship was constant over time, A_T can be estimated from reconstructions of salinity using sea-level records (Foster, 2008; Hönisch et al., 2009). However, salinity and alkalinity may be decoupled in space and time through weathering and changes in riverine alkalinity input. In addition, reliable proxies for regional salinity reconstructions have yet to be developed. Another approach is based on the assumption that seawater $[\text{Ca}^{2+}]$ has remained proportional to A_T over time so that A_T can be adjusted in a way that the water column is exactly saturated with respect to calcite at the lysocline (~ 500 m above the CCD; Pearson and Palmer, 2000). Surface A_T can be estimated by assuming that increases in A_T with depth were the same as in the modern ocean. The CCD, however, is not uniform through space and time (Van Andel, 1975), calling into question these approaches for estimating past A_T . Pearson and Palmer (2000) note that “the CCD record for the Palaeogene Pacific Ocean is relatively poorly constrained”.

Although $\delta^{11}\text{B}$ has proven to be a reliable proxy for pH and one can argue that ocean pH is the main driver of past atmospheric CO_2 , it is important to remember that changes in

past glacial interglacial atmospheric $p\text{CO}_2$ can be achieved via two end-member scenarios (e.g. Lea et al., 1999; Sanyal and Bijma, 1999). In the first scenario, changes in carbonate chemistry are brought about by changes in total dissolved inorganic carbon (C_T) only. This is equivalent to varying the response of the biological pump as a reaction to variations in the nutrient content of the surface ocean. In the second scenario, changes in carbonate chemistry are solely controlled by addition (due to dissolution in sediments) or removal (due to production) of calcium carbonate. The change in surface ocean carbonate chemistry is very different in these two scenarios because the ratio of carbonate ion increase in relation to $p\text{CO}_2$ decrease, depending on surface ocean alkalinity (Lea et al., 1999). A smaller change is associated with the drawdown of C_T under conditions of unchanging alkalinity (e.g. strengthening the biological pump without calcite compensation). The change in surface water $[\text{CO}_2]$ is twice as much when the same atmospheric $p\text{CO}_2$ is reached solely via a change in alkalinity as in the coral reef hypothesis (Lea et al., 1999). These dependencies are nicely demonstrated in Fig. 1.1.3 of Zeebe and Wolf-Gladrow (2001) and Fig. 1 of Foster and Rae (2016).

The real ocean operates somewhere between these end member scenarios and, basically, depends on the relative delivery rates of calcium carbonate and particulate organic carbon (the CaCO_3 :POC “rain ratio”) and the sensitivity of calcium carbonate preservation in deep ocean sediments. This demonstrates that a second, pH-independent, parameter could reduce the uncertainty in CO_2 estimates. On the other hand, the propagated uncertainty in the second parameter, reconstructed from an independent proxy (taking into account measurement and calibration uncertainty) might not be much lower than the margin of error that is garnered using assumptions around, for example, total alkalinity.

The boron isotope pH proxy in foraminifera has recently been reviewed by Foster and Rae (2016) and we refer to that for further reading. Here, we will briefly explain the boron systematics. Boron exists in seawater primarily in the form of two species, boric acid ($\text{B}(\text{OH})_3$) and borate ion ($\text{B}(\text{OH})_4^-$; Fig. 1a). As for all weak acids, the relative abundance between these two species is controlled by pH (Dickson, 1990; DOE, 1994). At low pH (< 7), nearly all boron is present in the form of boric acid, whereas at high pH (> 10), boron primarily exists as borate. Because of the isotopic fractionation between the two aqueous species (Fig. 1b; $\alpha_{4-3} = R_{\text{B}(\text{OH})_4^-} / R_{\text{B}(\text{OH})_3}$), the boron isotopic composition of each species is also pH-dependent (Hemming and Hanson, 1992; Palmer et al., 1987; Sanyal et al., 1996, 2000). $\text{B}(\text{OH})_3$ is enriched in the stable isotope ^{11}B compared to $\text{B}(\text{OH})_4^-$, with a constant isotopic fractionation of 27.2‰ between the two boron species (Klochko et al., 2009, 2006). Consequently, as the relative concentration of the dissolved species changes with pH, so does their isotopic composition. Because it is assumed that only the charged species, borate, is

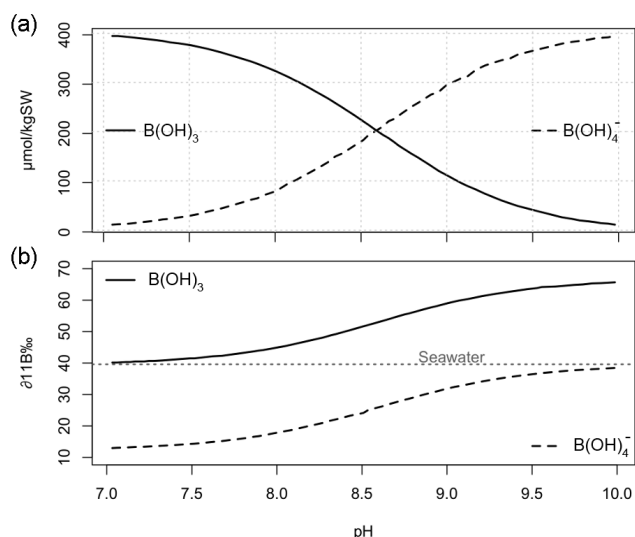


Figure 1. (a) Bjerrum plot showing the effect of pH on concentration of dissolved boron species at $T = 25\text{ }^\circ\text{C}$, $S = 35$ and $[\text{B}]_{\text{total}} 416\ \mu\text{mol kg}^{-1}$. (b) Effect of pH on boron isotopic composition of B(OH)_4^- and B(OH)_3 with thermodynamic fractionation factor (α_{3-4}) = 1.0272 (Klochko et al., 2006).

incorporated into the calcite lattice (Hemming and Hanson, 1992; Vengosh et al., 1991), the boron isotopic composition of marine carbonates thus records the pH that prevailed when the calcium carbonate was precipitated.

However, several studies have questioned the exclusive uptake of borate into calcite. For instance, Uchikawa et al. (2015) used inorganic precipitation experiments to show indirect evidence for incorporation of both B(OH)_4^- and B(OH)_3 into calcite. Based on first-principles quantum mechanical tools, Balan et al. (2016) concluded that the mechanisms of boron incorporation into calcium carbonates are probably more complex than assumed (i.e. not just charged borate). Although not invalidating the empirical paleo-pH proxy, their results call for a better understanding of the fundamental mechanisms of boron incorporation in carbonates. This demonstrates again that there is an urgent need for experiments where the primary controls of boron incorporation are investigated.

Considering the uncertainties associated with the constraints of $\delta^{11}\text{B}$ -based $p\text{CO}_2$ reconstructions, it is desirable to develop proxies for a carbonate system parameter in addition to pH. The B/Ca ratio of planktonic foraminifera has been proposed as a proxy for estimating past changes in $[\text{CO}_3^{2-}]$ (Foster, 2008); however, given that the concentration of borate B(OH)_4^- increases with pH and pH co-varies with $[\text{CO}_3^{2-}]$, it is challenging, if not impossible, to identify the parameter controlling B/Ca based on samples that have grown in natural seawater because pH and carbonate chemistry parameters co-vary closely in natural systems. To disen-

tangle their effects it is necessary to deconvolve the carbonate chemistry.

Such a study was recently carried out (Allen et al., 2012) and has shown that the B/Ca ratio of planktonic foraminifera also decreases with increasing total inorganic carbon (C_T or $[\text{HCO}_3^-]$) at constant pH (i.e. $[\text{B(OH)}_4^-]$ was constant while $[\text{CO}_3^{2-}]$ and $[\text{HCO}_3^-]$ were increased), suggesting that borate and carbon species compete for the inclusion in the calcite lattice. In their experiments, they kept pH constant and varied $[\text{CO}_3^{2-}]$ but did not vary pH at constant $[\text{CO}_3^{2-}]$, leaving the question open as to whether the B/Ca ratio in planktonic foraminifera is only a function of the ratio between $[\text{B(OH)}_4^-]$ and C_T or $[\text{HCO}_3^-]$ or perhaps also modulated by pH or $[\text{CO}_3^{2-}]$. Kaczmarek et al. (2015b) decoupled the carbonate chemistry both ways and showed that B/Ca in the benthic foraminifer *Amphistegina lessonii* is influenced by the ratio between $[\text{B(OH)}_4^-]$ and $[\text{HCO}_3^-]$, rather than by pH or $[\text{CO}_3^{2-}]$.

Recently, Henehan et al. (2015) demonstrated a very clear and close relationship between B/Ca and carbonate chemistry parameters (pH; $[\text{B(OH)}_4^-]/[\text{HCO}_3^-]$ and $[\text{B(OH)}_4^-]/C_T$) in *Globigerinoides ruber* from culture experiments. However, this relationship was completely lost in the plankton tow samples and the sediments they analysed. While they explicitly tested for a carbonate chemistry control on B/Ca, they found a strong relationship to $[\text{PO}_4^-]$ and neither a correlation with carbonate system parameters nor a covariation of phosphate with carbonate system parameters. They concluded that apparently B/Ca in *G. ruber* is controlled by $[\text{PO}_4^-]$. We will discuss why we believe that the primary (mechanistic) relationship explaining B/Ca is probably still controlled by carbonate chemistry parameters in the ambient environment of the foraminifer, but that it may be masked in the field and decoupled from the bulk seawater carbonate chemistry.

Here, we are specifically focussing on the primary controls of boron uptake and conducted experiments with the planktonic foraminifer *Orbulina universa* and decoupled pH and $[\text{CO}_3^{2-}]$ in the same way as Kaczmarek et al. (2015b). We show, in principle, that combined measurements of $\delta^{11}\text{B}_{\text{calcite}}$ and B/Ca on single shells of planktonic foraminifera might be used to fully constrain the carbonate chemistry in down-core records. However, based on recent publications (Allen et al., 2012; Babila et al., 2014; Henehan et al., 2015; Salmon et al., 2016), it becomes increasingly clear that in the field and downcore, B/Ca may not be a very robust carbonate system proxy (at least in some species) as the primary relationship can be masked by other environmental factors.

2 Methods

2.1 Collection and culturing

Living specimens of *O. universa* were collected daily using a 57 cm diameter WP2 plankton net (200 µm mesh size), between July and September 2012 at Point B, Villefranche-sur-Mer, France (43.41° N, 7.19° E), and maintained until gametogenesis in laboratory cultures at the Laboratoire d'Océanographie de Villefranche. Established procedures for maintaining planktonic foraminifera in laboratory culture were used (Bemis et al., 1998; Bijma et al., 1998; Spero and Lea, 1993). Briefly, specimens were identified, the diameters measured with a light microscope, and they were then transferred to 0.2 µm-filtered seawater, whose carbonate chemistry was accurately determined and subsequently modified. Specimens were maintained individually in airtight 100 mL acid-washed SCHOTT DURAN® bottles that were sealed without an air space and placed upside down into thermostated water baths maintained at a temperature of 23 °C (±0.2 °C). Light was provided by four 39 W fluorescent tubes (JBL Solar Ultra Marin Day), with reflectors (at a distance of ca. 15 cm from the water surface), with a 12:12 h L:D photoperiod. The average irradiance, measured with a LI-193 sensor (LiCOR) in the culture jars was about 290 µmol photons m⁻² s⁻¹.

The foraminifera were fed a one-day-old brine shrimp *Artemia* nauplius every second day until gametogenesis. The brine shrimp were hatched in modified seawater from the same batch as used for culturing the foraminifera. Just prior to feeding, hatched nauplii were transferred once again to fresh medium from the same batch. After feeding, culture jars were topped up with medium from the same batch to prevent the formation of a headspace. Empty shells were collected within 24 h after successful gametogenesis, rinsed in deionized water and archived in covered micro-paleo-slides for later analysis. Prior to analysis, specimens were harvested, bleached in NaOCl (active chlorine: 4.6 %) for 6 h, rinsed four times using deionized water, and dried for 12 h at 50 °C. Approximately 35 tests were grown for each experimental treatment. Culture water samples were collected at the start and end of the experiments to verify the boron concentration, its isotopic composition and the carbonate system parameters.

2.2 Modified seawater chemistry

The objective of these experiments was to decouple seawater pH and [CO₃²⁻] and create treatments with a constant pH and varying carbonate ion concentration and treatments with a constant carbonate ion concentration but varying pH. To decouple the effects of pH_T and [CO₃²⁻], seawater carbonate chemistry was modified by manipulating pH_T, using NaOH and HCl, and dissolved inorganic carbon (C_T) by adding gravimetrically carbonate and bicarbonate or bub-

bling with CO₂. Calculations were made using csys_vari.m (Zeebe et al., 2001) with carbonic acid dissociation constants of Mehrbach et al. (1973). Temperature (23 °C) and salinity (38.0) were kept constant (Table 1).

To enable single-shell analysis by LA-MC-ICP-MS, the boron concentration was increased to 10 times the concentration of natural seawater by adding boric acid to the culture water (see Sanyal et al., 2001, 2000). The pH_T and C_T were then modified via titration with boron-free NaOH (1N) and HCl (1N) to bring the experimental pH to desired levels of 7.70 ± 0.03, 7.90 ± 0.02, and 8.05 ± 0.05. Culture water samples collected at the start and at the end of each experiment showed that pH remained nearly constant throughout each experiment. The boron isotopic composition of each culture treatment is provided in Table 1. The pH of the culture solutions was measured using a Metrohm, 826 mobile pH meter with a glass electrode (Metrohm, electrode plus) calibrated to the total scale using TRIS and 2-aminopyridine buffer solutions (Dickson et al., 2007) adjusted to a salinity of 38.0. Total alkalinity (A_T) samples (150 mL) were filtered on GF/F and measured potentiometrically using a Metrohm Tritando 80 titrator and a Metrohm, electrode plus glass electrode (Dickson et al., 2007). 60 mL samples were also taken at the start and end of incubations and poisoned with 10 µL of saturated HgCl₂ pending determination of dissolved inorganic carbon (C_T). Samples were measured using an AIR-ICA (Marianda, Kiel) fitted with a Licor 6262 infrared gas analyser. All parameters of the carbonate system were calculated from A_T and pH_T (Hoppe et al., 2012) using the R package seacarb (Lavigne and Gattuso, 2013).

2.3 Culture water analysis

Boron isotopic composition of the culture media were analysed by means of a Thermo® Element XR, a single collector, sector field, high-resolution inductively coupled plasma mass spectrometer, fitted with a high-sensitivity interface pump (Jet pump) as described in Misra et al. (2014). Boron isotopic composition is reported as per mil (‰) deviation from NIST SRM 951a (¹¹B / ¹⁰B = 4.04362 ± 0.00137) (Catanzaro et al., 1970) where:

$$\delta^{11}\text{B}_{\text{sample}}(\text{‰}) = \left[\frac{(^{11}/^{10}\text{B})_{\text{sample}}}{(^{11}/^{10}\text{B})_{\text{NIST SRM951a}}} - 1 \right] \times 1000.$$

Boron isotope analyses were made following a Sample–Standard Bracketing (SSB) technique. NIST 951a was used as the standard and samples were concentration-matched, typically at 5 %, with the standard and were analysed in quintuplicate. The accuracy and precision of the analytical method was assessed by comparing ¹¹B measurements of seawater (from the Atlantic Ocean) and secondary boron standards (AE 120, 121, 122) with published (accepted) results. Our estimates of δ¹¹B_{SW} of 39.8 ± 0.4 ‰ (2 SE, n = 30) are independent of sample size and are in agreement

Table 1. Average properties of the manipulated seawater culture medium from four samples (two from the start of the incubation and two from the end of the incubation).

pH _T	C _T ($\mu\text{mol kg}^{-1}$)	A _T ($\mu\text{mol kg}^{-1}$)	pCO ₂ (μatm)	CO ₃ ²⁻ ($\mu\text{mol kg}^{-1}$)	HCO ₃ ⁻ ($\mu\text{mol kg}^{-1}$)	T (°C)	S	$\delta^{11}\text{B}$ (‰)
8.05 ± 0.02	2235.9	2566.8 ± 11	431.8	238.7	1981	23 ± 0.7	38 ± 0.6	5.35 ± 0.53
8.05 ± 0.05	2671.5	3050 ± 27	516.5	285.6	2370.6	23 ± 0.7	38 ± 1.02	4.98 ± 0.85
8.05 ± 0.03	4985.4	5594.3 ± 38	1103.7	533.9	4424.2	23 ± 0.7	38 ± 0.5	4.20 ± 1.03
7.9 ± 0.02	3809.2	4153.2 ± 154	1061	296.6	3478.4	23 ± 0.7	38 ± 0.3	4.11 ± 0.94
7.7 ± 0.03	5119.8	5361.8 ± 23	2335.1	257.8	4791.6	23 ± 0.7	38 ± 0.9	4.69 ± 2.4

with published values of 39.6 ± 0.04 ‰ (2 SE, $n = 28$) (Foster et al., 2010) and 39.5 ± 0.6 ‰ (2 SD) (Spivack and Edmond, 1987). Moreover, our $\delta^{11}\text{B}$ estimates of SRM AE-120 (-20.2 ± 0.5 ‰, 2 SE, $n = 33$), SRM AE-121 (19.8 ± 0.4 ‰, 2 SE, $n = 16$), SRM AE-122 (39.6 ± 0.5 ‰, 2 SE, $n = 16$) are identical, within analytical uncertainty, to accepted values (Vogl and Rosner, 2012). Information about sample preparation for analysis can be found in the Supplement provided in Kaczmarek et al. (2015a).

2.4 Analysis of *O. universa*

For simultaneous determination of the B isotopic composition and its concentration, a Fiber Optics Spectrometer (Maya2000 Pro, Ocean Optics) was connected to the torch of a Thermo Finnigan Neptune multiple-collector inductively coupled plasma mass spectrometer (MC-ICP-MS) at the Leibniz University of Hannover. Laser ablation on reference material and samples was performed by an in-house-built UV-femtosecond laser ablation system based on a regenerative one-box femtosecond laser (Solstice Newport/Spectra Physics). A detailed description of the method used for the simultaneous determination of B concentration and $\delta^{11}\text{B}$ of *O. universa* can be found in Kaczmarek et al. (2015a). A summary of the procedure is given below.

2.5 Simultaneous determination of B concentration and $\delta^{11}\text{B}$

The B intensity of a reference material corresponds to its known B concentration. Based on this relationship, the unknown B concentration of a sample can be calculated. However, our measurements of the reference material (NIST SRM 610) and samples were not performed at the same laser repetition rate: hence, their B ratios are not proportional. Because Ca concentrations in the reference material and in the sample are known (NIST SRM 610: 8.45 %, CaCO₃: 40 %) a correction for different laser repetition rates was realized by the analysis of calcium using the optical spectrometer. More information on this procedure is provided by Longerich et al. (1996).

2.6 Calcium analysis

The Maya2000 Pro is a high-sensitivity fiber optical spectrometer. It has a measuring range between 250 and 460 nm with a resolution of 0.11 nm covering the first order emission lines of Mg II, Ca II, Sr II, Ba II and Li II. It is equipped with a back-thinned 2-D FFT-CCD detector, and a grating with a groove density of 1200 lines mm⁻¹. The optical fiber used is 2 m long (attenuation of the photon flux is length-dependent), connecting the spectrometer with the coupling lens at the end of the plasma torch of the MC-ICP-MS (Thermo Finnigan Neptune). Ca II ion lines were measured at a wavelength of 393.48 and 396.86 nm. At these wavelengths the Ca spectrum shows no detectable interferences for the matrices used. The acquisition parameters were set to acquire 220 cycles per analysis with an integration time of 1 s for each cycle. For the first 40 cycles, only background (BG) signal was detected prior to measuring the sample. The BG signal detected at the start of the analysis was later used for correcting sample measurements by subtracting BG intensity from the intensity of the reference and the sample material.

2.7 Boron isotope analysis – 194 nm femtosecond laser ablation

The in-house-built laser ablation system is based on a 100 femtosecond Ti-sapphire regenerative amplifier system operating at a fundamental wavelength of 777 nm in the infrared spectrum. Subsequent harmonic generations produce the wavelengths 389 nm in the second, 259 nm in the third and 194 nm in the fourth harmonic. The pulse energies measured with a pyroelectric sensor (Moletron, USA) are 3.2 mJ pulse⁻¹ at 777 nm, 0.7 mJ pulse⁻¹ at 259 nm, and 0.085 mJ pulse⁻¹ at 194 nm. After the fourth harmonic generation stage, the 194 nm beam is steered by eight dichroic mirrors into an 8 × objective (NewWave Research, USA) and focussed onto the outside of the sample. Spot size was set to 50 μm for the reference material and the samples. Within this spot, an energy density of $\sim 2 \text{ J cm}^{-2}$ is maintained. Reference material measurements were performed in raster mode (100 $\mu\text{m} \times 100 \mu\text{m}$) at 10 Hz and samples were ablated at 8–50 Hz depending on B concentration.

It should be noted that the fs laser ablation process is fundamentally different from ns laser ablation. When the pulse length is shorter than 1 ps (Hergenröder et al., 2006), the laser energy can be deposited into the material before it can thermally equilibrate. Femtosecond ablation also provides smaller aerosol particle sizes. Due to the short pulse length, fs laser ablation shows no detectable matrix dependency (e.g. Chmeleff et al., 2008; Horn et al., 2006; Kaczmarek et al., 2015a; Lazarov and Horn, 2015; Oeser et al., 2014; Schuessler and von Blanckenburg, 2014), i.e. it does not require a matrix-matched standard and therefore permits the use of NIST SRM 610 (a glass) as a reference for carbonates. As boron concentrations differ between sample and standard, and different matrices require more or less energy for ablation, the repetition rate was chosen such that the signal of sample and standard at the ion counters was comparable. This is important for normalization of the sample to the known $\delta^{11}\text{B}$ of the standard and also accounts for the imprecision of the determined detector dead time.

Most previous publications on boron isotopes have used “wet chemistry” for which NIST SRM 951 is a perfect standard. We have also used this standard for the analysis of the culture waters. The foraminiferal shells, however, were referenced against NIST SRM 610. As shown by several studies (Fietzke et al., 2010; Kasemann et al., 2001; Le Roux et al., 2004), both standards are, within analytical uncertainty, isotopically equal. Hence, for comparison between $\delta^{11}\text{B}$ of *O. universa* and $\delta^{11}\text{B}$ of $\text{B}(\text{OH})_4^-$, the isotopic difference between the two standards can be neglected and it does not make a difference if values are reported vs. one or the other standard.

2.8 Boron isotope analysis – acquisition parameters

All measurements are carried out in low mass resolution ($\Delta m/m = 350$ where m is the mass of the ion of interest and Δm is the mass difference between its 5 and 95 % peak height). Compact discrete dynode multipliers (CDDs, Thermo) are attached to Faraday cups at the low site on L4 and the high site on H4. The low-resolution mode is sufficient to resolve potential interferences from doubly charged ions due to the intrinsic high resolution in the low mass region. Possible interferences are the clusters of $^{40}\text{Ar}^{4+}$ or $^{20}\text{Ne}^{2+}$, which are well resolved to the background level. The instrument was tuned prior to each analytical session for optimal peak shape. Instrumental operating conditions are reported in Table 2. All measurements were performed at plateau voltage of the CDDs, which was checked prior to every analytical session. Before the beginning of sample analysis, measurements of NIST SRM 610 were continued until instrumental drift (due to warm-up) was less than 200 ppm over a bracketing sequence duration of twelve minutes. Boron signal intensities of NIST SRM 610 and samples were matched within 10 % in signal intensity by adapting the laser repetition rate. The acquisition parameters in static mode for analysis of

Table 2. Instrumental operating conditions for the MC-ICP-MS and LA.

Cool Gas [L min^{-1}]:	14.6
Aux Gas [L min^{-1}]:	1.2
Sample Gas [L min^{-1}]:	1.5
Add Gas [L min^{-1}]:	0.4
Operation Power [W]:	1269
X Pos [mm]:	1.5
Y Pos [mm]:	−1.7
Z Pos [mm]:	−2.5
Wavelength [nm]	194
Pulse energy [J cm^{-2}]	2
Pulse width [fs]	~ 200
Spot size [μm]	50

NIST SRM 610 and samples were set to acquire 200 cycles of 1 s integrations each. During the first 40 cycles the background signal was acquired, whereas the remaining cycles represent the sum of the background and the reference material, or the background and the sample signals. A complete measurement consisting of 200 cycles of a single reference material or sample took 4 min before the next sample was introduced. For analysis we adopted the standard sample bracketing procedure and the B isotopic composition is reported using the delta notation:

$$\delta^{11}\text{B}_{\text{sample}}(\text{‰}) = \left[\frac{(^{11}\text{B}/^{10}\text{B})_{\text{sample}}}{\left((^{11}\text{B}/^{10}\text{B})_{\text{NIST610-1}} + (^{11}\text{B}/^{10}\text{B})_{\text{NIST610+1}} \right) / 2} - 1 \right] \times 1000, \quad (1)$$

where NIST 610 – 1 and NIST 610 + 1 refer to the analysis of the reference material before and after the sample. The uncertainty of the samples was calculated according to:

$$2\text{SE}_{\delta^{11}\text{B}_{\text{sample}}}(\text{‰}) = \sqrt{\left(\frac{\text{SE}}{(^{11}\text{B}/^{10}\text{B})_{\text{NIST-1}}} \right)^2 + \left(\frac{\text{SE}}{(^{11}\text{B}/^{10}\text{B})_{\text{sample}}} \right)^2 + \left(\frac{\text{SE}}{(^{11}\text{B}/^{10}\text{B})_{\text{NIST+1}}} \right)^2} \times 2 \times 1000, \quad (2)$$

where $^{11}\text{B}/^{10}\text{B}$ ratios represent mean values of the reference material and the sample calculated from one measurement (based on 160 cycles) and SE represents the standard error of the $^{11}\text{B}/^{10}\text{B}$ ratios. Due to the natural inhomogeneity of the samples, the analytical uncertainty is represented best by repeated measurements of the homogenous reference material

given by:

$$\delta^{11}\text{B}_{\text{NIST610}}(\text{‰}) = \left[\frac{{}^{11/10}\text{B}_0}{({}^{11/10}\text{B}_{-1} + {}^{11/10}\text{B}_{+1})/2} - 1 \right] \times 1000, \quad (3)$$

where the measurements of the $({}^{11/10}\text{B})_{-1}$ and $({}^{11/10}\text{B})_{+1}$ ratios of NIST 610 were performed before and after the measurement of $({}^{11/10}\text{B})_0$, respectively. For the determination of the analytical uncertainty and external reproducibility, all measurements of NIST 610 performed between each sample measurement were taken into account. On average, the analytical uncertainty and external reproducibility is 0.66‰.

2.9 Conversion of $\delta^{11}\text{B}_{O. universa}$ to natural seawater

Due to the additional B addition to our culture media the $\delta^{11}\text{B}_{\text{seawater}}$ shifted from 37.63 (Mediterranean) to, on average, 4.66‰ (Table 1). Therefore, the $\delta^{11}\text{B}_{O. universa}$ shifted accordingly. In order to compare our *O. universa* data to published values (Fig. 3a), the measured $\delta^{11}\text{B}$ from each experiment was normalized to natural seawater using the following (Zeebe and Wolf-Gladrow, 2001):

$$\delta^{11}\text{B}_c = \alpha_{\text{sw-msw}} \times \delta^{11}\text{B}_m + \varepsilon, \quad (4)$$

where ε is $(\alpha_{\text{sw-msw}} - 1) \times 1000$, $\delta^{11}\text{B}_c$ represents the converted $\delta^{11}\text{B}$ for the measured value ($\delta^{11}\text{B}_m$), $\alpha_{\text{sw-msw}}$ is the fractionation factor expressing the difference between the natural seawater and manipulated seawater:

$$\alpha_{\text{sw-msw}} = \left(\delta^{11}\text{B}_{\text{sw}} + 10^3 \right) / \left(\delta^{11}\text{B}_{\text{msw}} + 10^3 \right). \quad (5)$$

2.10 Statistics

Lamtool (a modified Excel spreadsheet, initially programmed by Jan Kosler, University of Bergen, Norway) was used for analysis and background correction of the $\delta^{11}\text{B}$ data. All other statistics were carried out using R (R Core Team, 2008). Error bars represent $\pm 2\sigma$ errors, correlations were calculated by linear regression. The procedures for data evaluation, background correction and uncertainty calculations for boron concentration and isotopes are extensively described in Kaczmarek et al. (2015a).

In contrast to “wet chemical” analysis, laser ablation (LA) records the inhomogeneous boron distribution (“boron banding”, see Branson et al., 2015) within a specimen and individual shell analysis captures inter-specimen differences. Sadekov et al. (2016) demonstrated that the variability in both B/Ca and $\delta^{11}\text{B}$ recurs in each chamber and, therefore, represents real data of high quality. This is supported by the fact that the values of the averaged laser data are very close to wet chemical analyses where multiple specimens are dissolved and the intra- and inter-variability is “averaged” before the analysis. The intra-specimen $\delta^{11}\text{B}$ variability in

Cibicides wuellerstorfi is up to ca. 10‰ (Sadekov et al., 2016), while the inter-specimen $\delta^{11}\text{B}$ variability of *Amphistegina lessonii* from the same treatment is ca. 6‰ (Kaczmarek et al., 2015b). Histograms of single-foram $\delta^{11}\text{B}$ measurements from each of our pH treatments (Fig. S1 in the Supplement) show that the LA data is normally distributed (p values from Shapiro–Wilk tests are all higher than 0.05). This is confirmed by the box plots (Fig. S1) where the average and median values are very close to each other. The relatively large standard errors of laser ablation analyses are representative of true inter-specimen variability and largely unrelated to analytical errors. Therefore, the relatively large standard errors do not present a limitation for how much can be interpreted from the data. The major difference between LA and “wet chemistry” data is that the latter method averages individual variability before analysis by measuring multiple dissolved shells in one go, while LA captures individual variability (which is large and real as argued above) and averages afterwards.

One could further argue that the uncertainty stemming from the analysis of culture water $\delta^{11}\text{B}$ should also be propagated when plotting in “normal $\delta^{11}\text{B}_{\text{sw}}$ ” space (Table S4 in the Supplement). The propagated error is, of course, large as it includes the individual $\delta^{11}\text{B}$ variability of the foraminifers. It is important to acknowledge that this variability represents true data, which is largely unrelated to analytical uncertainty. We added a calcite vs. borate $\delta^{11}\text{B}$ cross-plot (Fig. 4) to avoid the conversion into the seawater scale and making the error propagation obsolete. However, as not all studies report the parameters required for the calculation of $\delta^{11}\text{B}$ of borate, we plotted for comparison in “normal $\delta^{11}\text{B}_{\text{sw}}$ ” space (Fig. 3a) but did not propagate the error related to the analysis of culture water $\delta^{11}\text{B}$.

3 Results

3.1 B / Ca ratios

The B / Ca ratio of *O. universa* shows a strong negative correlation ($R^2 = 0.96$) with C_T , irrespective of the pH_T of the culture media (Fig. 2a). It is also correlated to $[\text{CO}_2]$ but to a lesser extent ($R^2 = 0.64$; Fig. 2c). B / Ca also decreases with increasing $[\text{CO}_3^{2-}]$ in specimens grown under a pH_T of 8.05, (Fig. 2e). However, the B / Ca ratio of specimens grown under lower pH_T values (7.9 and 7.7) is negatively offset from the relationship found at pH_T 8.05 and the overall correlation of B / Ca and $[\text{CO}_3^{2-}]$ is very low ($R^2 = 0.2$; Fig. 2e). Of all the carbonate species, the B / Ca ratio exhibits the best negative relationship with increasing $[\text{HCO}_3^-]$, irrespective of the pH_T of the culture medium ($R^2 = 0.96$; Fig. 2g). Plotted against the ratio of $[\text{B}(\text{OH})_4^-]$, over each of the carbon species (Fig. 2b, d, f, h), the correlations are high for all combinations but highest for $[\text{B}(\text{OH})_4^-] / [\text{CO}_3^{2-}]$. Based on first principles, we predict a positive correlation between

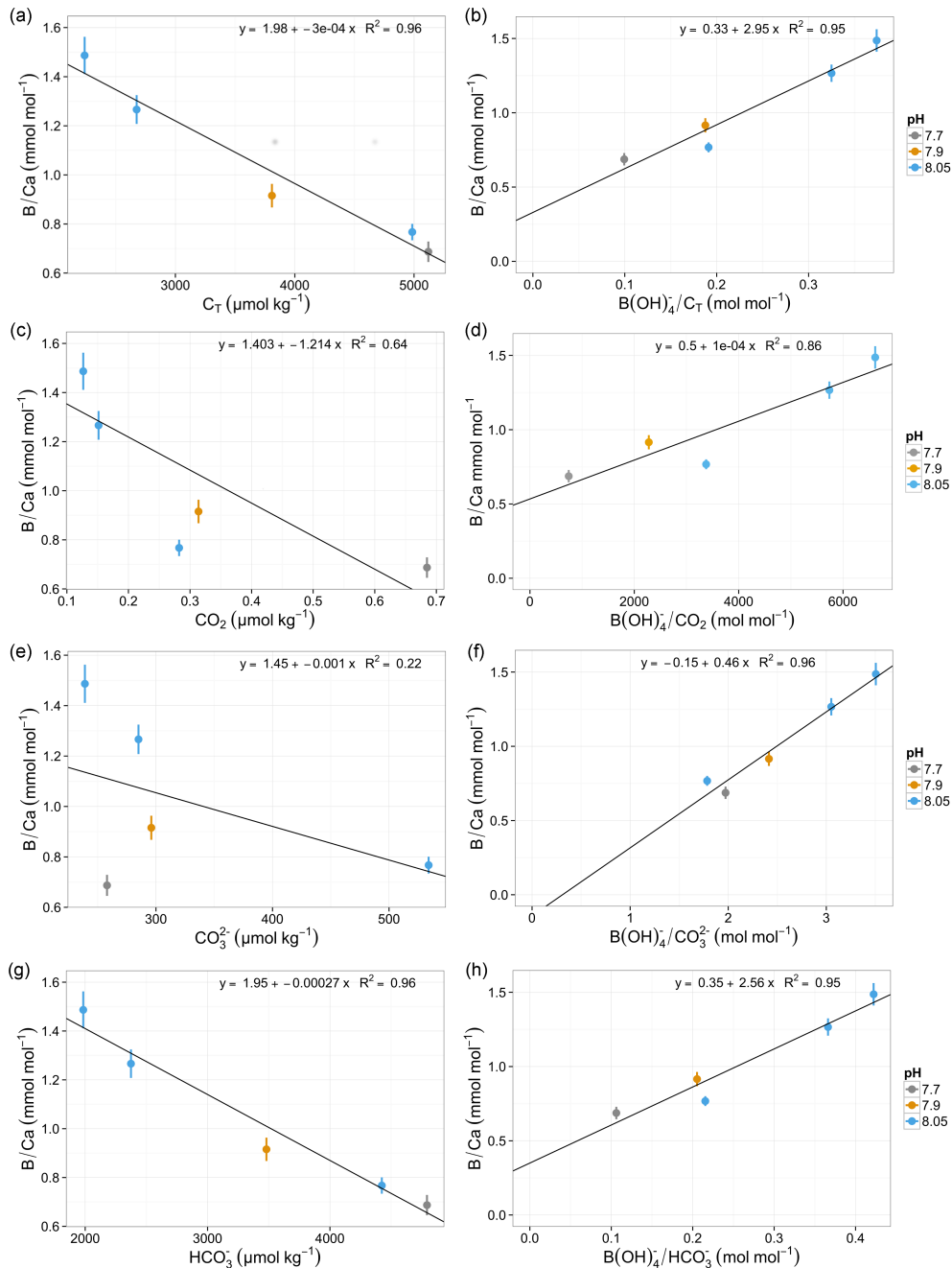


Figure 2. B/Ca ratios plotted against (a) [C_T], (b) [B(OH)₄⁻]/[C_T], (c) [CO₂], (d) [B(OH)₄⁻]/[CO₂], (e) [CO₃²⁻], (f) [B(OH)₄⁻]/[CO₃²⁻], (g) [HCO₃⁻], (h) [B(OH)₄⁻]/[HCO₃⁻], error bars represent standard error.

B/Ca and $\delta^{11}\text{B}$ because at higher pH, not only does the isotopic composition of borate become heavier, but its concentration also increases. Figure S2 shows the individual B/Ca, $\delta^{11}\text{B}$ pairs per treatment. As expected for individual LA shell analyses, the inter specimen variability is quite large. Individual B/Ca ratios vary by almost 50% in each treatment and individual $\delta^{11}\text{B}$ values vary by ca. 4–6‰ per treatment (cf. Kaczmarek et al., 2015b). Although one

could argue for a positive trend between B/Ca and $\delta^{11}\text{B}$ in some of the treatments, we believe that the individual B/Ca, $\delta^{11}\text{B}$ pairs within a treatment are uncorrelated. However, the average values for the four treatments with [CO₃²⁻] between 238–297 $\mu\text{mol kg}^{-1}$ do show a positive correlation between B/Ca and $\delta^{11}\text{B}$. The “outlier” (treatment at pH 8.05; [CO₃²⁻] = 534) can be explained by the high [HCO₃⁻], relative to the other pH = 8.05 treatments.

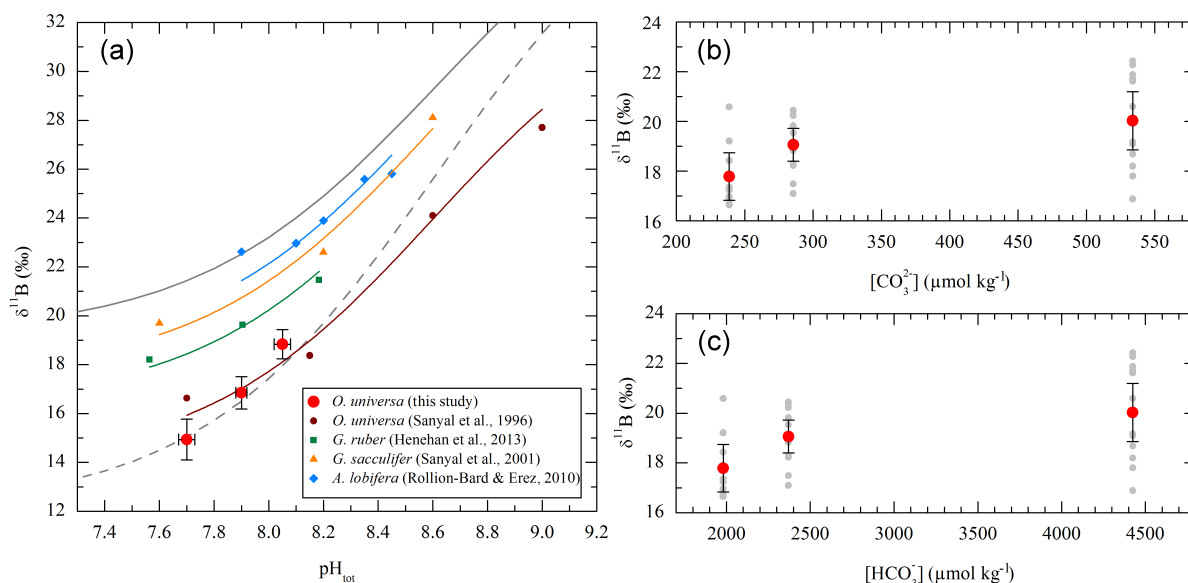


Figure 3. (a) Converted median $\delta^{11}\text{B}$ of cultured *O. universa* calcite (red circles) error bars represent $\pm 2\sigma$ errors, solid grey line shows empirical values for seawater $\delta^{11}\text{B}_{\text{borate}}$ with a fractionation factor of $^{11-10}K_{\text{B}} = 1.020$ (Hönisch et al., 2007) at $T = 23^\circ\text{C}$ and $S = 38$. Dashed grey line shows the experimental $\delta^{11}\text{B}_{\text{borate}}$ curve with a fractionation factor of $^{11-10}K_{\text{B}} = 1.0272$ (Klochko et al., 2006) at $T = 23^\circ\text{C}$ and $S = 38$. (b) Median $\delta^{11}\text{B}$ cultured *O. universa* calcite grown at constant pH of 8.05 but varying $[\text{CO}_3^{2-}]$. (c) Median $\delta^{11}\text{B}$ of $\text{B}(\text{OH})_4^-$ in cultured *O. universa* calcite grown at constant pH of 8.05 but varying $[\text{HCO}_3^-]$. Error bars represent $\pm 2\sigma$ errors across all single laser ablation analyses per treatment.

3.2 Boron isotopic fractionation ($\delta^{11}\text{B}$)

Single, measured $\delta^{11}\text{B}$ values of *O. universa* are given in Table S1 and errors are calculated according to Eq. (2). Median and converted values using Eqs. (4) and (5) are shown in Fig. 3a and Table 3. The fractionation of boron isotopes in the shells of *O. universa* is dependent on the pH of the culture medium, increasing with pH_{T} from 15‰ at $\text{pH}_{\text{T}} 7.7$ to 18.8‰ at $\text{pH}_{\text{T}} 8.05$. These values are close to the B fractionation curve of $\text{B}(\text{OH})_4^-$ obtained for artificial seawater by Klochko et al. (2006; Fig. 3a). $\delta^{11}\text{B}$ increases with increasing $[\text{CO}_3^{2-}]$ at constant pH_{T} from 17.2‰ at $238 \mu\text{mol kg}^{-1} \text{CO}_3^{2-}$ to 19.9‰ at $534 \mu\text{mol kg}^{-1} \text{CO}_3^{2-}$ (Table 3; Fig. 3b). Applying ANOVA with a Bonferroni test, which is best suited for a limited number of pairs, the p value of the overall ANOVA is 0.00203, demonstrating a significant difference between two or more population means. The differences between the mean $\delta^{11}\text{B}$ values of the $[\text{CO}_3^{2-}]$ treatments 239 and $286 \mu\text{mol kg}^{-1}$ were close to significance but only between 239 and $534 \mu\text{mol kg}^{-1}$; the difference was significant (Table S3). Because this range in $[\text{CO}_3^{2-}]$ is beyond that of the real ocean and because pH and $[\text{CO}_3^{2-}]$ covary, we believe that this observation is only important for a better understanding of the $\delta^{11}\text{B}$ controls and does not significantly impact existing calibrations.

4 Discussion

4.1 B / Ca

Foster (2008) showed that the partition coefficient K_{D} for the B / Ca ratio is influenced by $[\text{CO}_3^{2-}]$ (and temperature). Although complicating the application as a proxy related to $[\text{B}(\text{OH})_4^-] / [\text{HCO}_3^-]$, he also demonstrated that B / Ca in combination with $\delta^{11}\text{B}$ can be used to fully constrain the carbonate system in downcore records. Nonetheless, he identified $[\text{CO}_3^{2-}]$ as having a major (secondary) control on B / Ca in samples of foraminifera from down-core samples and core tops. A similar conclusion was reached by Allen et al. (2011) for *O. universa*. These authors demonstrated a trend of decreasing B / Ca with increasing pH and $[\text{CO}_3^{2-}]$; however, due to the co-variations of the carbonate system in natural seawater, it is difficult to identify the differential effects of the individual parameters. Allen and Hönisch (2012) conclude that the relationships between K_{D} and seawater parameters can sometimes be driven by the denominator of the empirical boron partition coefficient ($[\text{B}(\text{OH})_4^-] / [\text{HCO}_3^-]$), and not by B / Ca of seawater itself. Reconstructions based on such B / Ca-independent relationships are susceptible to being driven by other environmental parameters. They conclude that application of the empirical boron partition coefficient should be avoided until more is known about the relative influences of different chemical species on boron incorporation.

Table 3. Average (B / Ca) and average and median $\delta^{11}\text{B}$ values for the different experimental treatments.

pH _T	CO ₃ ²⁻ ($\mu\text{mol kg}^{-1}$)	Average $\delta^{11}\text{B}$ ± 2 s.e. (‰)	Median $\delta^{11}\text{B}$ ± 2 s.e. (‰)	B / Ca ± 2 s.e.	N samples
8.05 \pm 0.02	238.7	17.8 \pm 1.0	17.2 \pm 1.0	1.27 \pm 0.6	9
8.05 \pm 0.05	285.6	19.1 \pm 0.7	18.8 \pm 0.7	1.49 \pm 0.58	11
8.05 \pm 0.03	533.9	20.0 \pm 1.1	19.9 \pm 1.1	0.77 \pm 0.3	12
7.9 \pm 0.02	296.6	16.8 \pm 0.7	16.8 \pm 0.7	0.92 \pm 0.49	18
7.7 \pm 0.03	257.8	14.7 \pm 0.8	14.9 \pm 0.8	0.69 \pm 0.37	15

Experimentally decoupling pH_T from other parameters of the carbonate system using modified seawater media allowed us to decouple the relationships and identify the controlling carbon species. Our results demonstrate that the amount of boron incorporated into *O. universa* calcite is a function of C_T (Fig. 2a). As C_T increases, B / Ca decreases, suggesting that B(OH)₄⁻ competes with carbon species for inclusion in the calcite lattice. When B / Ca ratios are plotted against [CO₂], the relationship is similar to that of C_T; however, only < 1 % of C_T is in the form of CO₂ so this species is unlikely to have a major control on boron incorporation. The remaining > 99 % is ~ 10 % CO₃²⁻ and ~ 90 % HCO₃⁻ (Zeebe and Wolf-Gladrow, 2001). Due to the strong correlation of the B / Ca ratio and [B(OH)₄⁻] / [C_T], one could argue that foraminifera utilize both HCO₃⁻ and CO₃²⁻ as substrate for calcification and, therefore, that C_T is the factor controlling the B / Ca ratios. However, because [HCO₃⁻] and [CO₃²⁻] in our treatments increase and decrease with decreasing pH_T, respectively (Table 1), we can distinguish between bicarbonate and carbonate ion control over the B / Ca ratio.

At constant pH_T, the relationship between B / Ca and [CO₃²⁻] (Fig. 2e) supports the hypothesis of competition between CO₃²⁻ and B(OH)₄⁻. However, when [CO₃²⁻] is held constant and pH_T is decreased, B / Ca significantly decreases despite the fact that [CO₃²⁻] remains more or less constant (Fig. 2e, Table 1). If the same relationships are examined for B / Ca and [HCO₃⁻] a strong correlation between [HCO₃⁻] and B / Ca is observed for both the absolute concentration of HCO₃⁻ (Fig. 2g) and also for the ratio of [B(OH)₄⁻] / [HCO₃⁻] with no effect of changing pH_T (Fig. 2h). The close correlation between [CO₃²⁻] and B / Ca at constant pH_T can be explained by the corresponding increases in [HCO₃⁻] in these treatments (Table 1).

In agreement with our results, the study of Allen et al. (2012) investigated the effects of decoupling pH and the carbonate system on B / Ca and suggest that B(OH)₄⁻ competes with carbon species for inclusion in the calcite lattice in three planktonic species *Globigerinoides sacculifer*, *Globigerinoides ruber*, and *Orbulina universa*. Although analysis of planktonic foraminifera from core tops revealed a good correlation between B / Ca and [B(OH)₄⁻] / [HCO₃⁻] it

does not rule out a possible correlation with B(OH)₄⁻ / CO₃²⁻ and/or B(OH)₄⁻ / C_T (Yu et al., 2007).

A recent study by Kaczmarek et al. (2015b) shows the same competition between B(OH)₄⁻ and HCO₃⁻ in the benthic species *A. lessonii* cultured in a pH-[CO₃²⁻] decoupled seawater. The observation that B / Ca is driven by B(OH)₄⁻ / HCO₃⁻ and not related to CO₃²⁻ only becomes visible at higher pH (8.6) when [B(OH)₄⁻] is sufficiently high (see Fig. 6 and Table S1 in Kaczmarek et al., 2015b). Below pH 8.6, foraminiferal B / Ca also correlates with B(OH)₄⁻ / CO₃²⁻.

The finding that B(OH)₄⁻ / HCO₃⁻ controls boron incorporation in *O. universa* calcite is also in agreement with the hypotheses of Hemming and Hanson (1992) who suggested that only B(OH)₄⁻ is incorporated into marine carbonates with the partition coefficient defined below:

$$K_D = \frac{[\text{B} / \text{Ca}]_{\text{solid}}}{[\text{B}(\text{OH})_4^- / \text{HCO}_3^-]_{\text{seawater}}}. \quad (6)$$

To summarize, based on our study, we can eliminate a control by [CO₃²⁻] but cannot exclude [B(OH)₄⁻ / CO₃²⁻]. By comparison to the B / Ca control in the benthic foraminifer *A. lessonii* (Kaczmarek et al., 2015b), we assume B / Ca in planktonic foraminifera is also a function of [B(OH)₄⁻ / HCO₃⁻].

4.2 Boron isotopic fractionation ($\delta^{11}\text{B}$)

As the various species of inorganic carbon and pH_T are tightly linked, it is still to be experimentally demonstrated, beyond doubt, whether only pH_T and/or the concentration of one or several carbonate species might have an effect on $\delta^{11}\text{B}$. The results for treatments with varying pH_T and constant carbonate ion concentration displayed the same relationship as those from the calibration curve for *O. universa* produced by Sanyal et al. (1996) but the absolute values for a given pH_T are lower by approximately 1 to 2 ‰ when compared to the values corrected to the fractionation factor suggested by Klochko et al. (2006; Zeebe et al., 2008). The effects of the unnaturally high C_T and A_T values in the treatments cannot be discounted as the cause of this difference, as $\delta^{11}\text{B}$ values increased with increasing [CO₃²⁻]. The $\delta^{11}\text{B}$ values for *O. universa* found in this study match closely with

the $\delta^{11}\text{B}$ values of borate ion in artificial seawater given by Klochko et al. (2006). This is probably caused by the suppression of the vital effects imposed by *O. universa*. Theoretical considerations demonstrate that at $10\times$ boron concentration compared to natural seawater, vital effects are suppressed and the isotopic value of biogenic calcite approaches the value of borate (Zeebe, 2003). This was confirmed by the comparison of the boron isotopic values of *O. universa* grown at low and high light (Hönisch et al., 2003) and supports the notion that borate is, indeed, the species being taken up. There is a trend of varying $[\text{CO}_3^{2-}]$ on $\delta^{11}\text{B}$ of samples grown at the same pH but, most importantly, in light of the results obtained for the B/Ca ratio, there is no effect of $[\text{HCO}_3^-]$ (Fig. 3c).

4.3 Proxy implications

A sound understanding of the effects of past carbon perturbations becomes increasingly urgent in an age where anthropogenic activities are producing such rapid changes in global climate (Bijma et al., 2013; Knoll and Fischer, 2011). The usefulness of biogeochemical proxies to reconstruct paleo-oceanographic conditions is well established for many environmental parameters (Wefer et al., 1999) but uncertainties remain for proxies related to pH and the carbonate system (Allen and Hönisch, 2012; Hönisch et al., 2007; Katz et al., 2010; Pagani et al., 2005). This study confirms the robustness of $\delta^{11}\text{B}$ as an independent pH proxy and supports the growing body of evidence that B/Ca in planktonic foraminiferal calcite is mechanistically controlled by $[\text{HCO}_3^-]$ (Yu et al., 2007), thereby allowing researchers to fully constrain the carbonate system in combination with $\delta^{11}\text{B}$.

Based on our results and other culture studies, it becomes clear that despite strong biological effects on the ambient carbonate chemistry (Köhler-Rink and Köhl, 2001, 2000; Rink et al., 1998; Wolf-Gladrow et al., 1999; Zeebe et al., 2008), the boron isotopic composition and the B/Ca are faithful predictors of seawater pH and bicarbonate ion concentration, respectively. Our results provide strong evidence that $[\text{HCO}_3^-]$ is the primary control of the B/Ca ratio. The correlation of the B/Ca ratio to $[\text{HCO}_3^-]$ rather than to $[\text{CO}_3^{2-}]$ might have some implications for existing paleo-carbonate chemistry reconstructions based on this proxy such as the study by Foster (2008) and that of Yu et al. (2014), since it seems reasonable to assume that the same relationship probably holds for benthic foraminifers as for planktonics.

A wide range of $[\text{HCO}_3^-]$ was necessary to facilitate decoupling the carbonate system from pH_T . The high $[\text{HCO}_3^-]$ in some of these treatments are unrealistic for natural seawater systems and more environmentally-relevant values should be used for future calibration experiments. The proxy should, therefore, be ground-truthed using water column and core top samples.

Recently, Henehan et al. (2015) showed that B/Ca in *G. ruber* collected with a plankton net was perfectly corre-

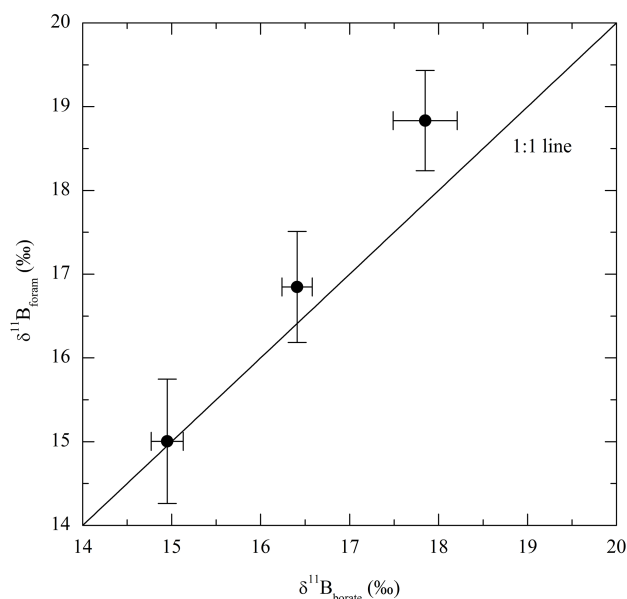


Figure 4. $\delta^{11}\text{B}$ of borate vs. that of calcite. $\delta^{11}\text{B}_{\text{borate}}$ was calculated using the fractionation factor of $^{11-10}K_{\text{B}} = 1.0272$ (Klochko et al., 2006) at $T = 23^\circ\text{C}$ and $S = 38$. Error bars for $\delta^{11}\text{B}_{\text{foram}}$ represent $\pm 2\sigma$ errors, and for $\delta^{11}\text{B}_{\text{borate}}$ the translated uncertainty of pH measurements (Table 3).

lated to $[\text{PO}_4^{3-}]$ and not to any carbonate chemistry parameter, despite the fact that their culture study demonstrated a highly significant relationship between B/Ca and for example $[\text{B}(\text{OH})_4^-]/[\text{HCO}_3^-]$. Based on plankton tow, sediment trap and core-top data, they concluded that, apparently, B/Ca in *G. ruber* is controlled by $[\text{PO}_4^{3-}]$. However, it should be noted that foraminifera, and especially symbionts bearing planktonic foraminifers, never experience the bulk ocean carbonate chemistry (e.g. micro-electrode study by Rink et al., 1998, and the modelling study by Wolf-Gladrow et al., 1999). They only experience their ambient carbonate chemistry as modulated by their own life processes, symbiont photosynthesis and respiration (so called “vital effects”). Existing calibrations and field relationships are therefore purely empirical and not mechanistic and the ambient carbonate chemistry of the foraminifer and the bulk seawater chemistry can be decoupled. We note that, of all symbionts bearing planktonic foraminifera, *G. ruber* is probably the most “autotrophic” (Bijma et al., 1992). Although we cannot prove it, we assume that symbiont photosynthetic rates are higher at elevated $[\text{PO}_4^{3-}]$ (limiting nutrient) and therefore that ambient pH would be higher. Hence, even if the correlation between B/Ca and seawater carbonate chemistry is lost, the correlation with ambient pH (or $[\text{CO}_3^{2-}]$, $[\text{HCO}_3^-]$) may still hold up. At this point, there is no obvious direct link between $[\text{PO}_4^{3-}]$ and B/Ca and we believe that, mechanistically, it can be explained by increased photosynthesis and/or higher calcification rates. Kaczmarek et al. (2016) show that

boron partitioning in inorganic precipitation experiments increases with increasing growth rate, and early work by the pioneers of foraminiferal biology and calcification (e.g. Bé et al., 1982; Bé, 1965; Caron et al., 1982; Hemleben et al., 1987; Jørgensen et al., 1985; Spero and Parker, 1985) clearly demonstrated the huge impact of symbionts on foraminiferal shell growth. Interestingly, Babila et al. (2014) state: “The seasonal cycle of B / Ca in *G. ruber* white was more strongly correlated with light intensity than with temperature. Both observations suggest that the presence of symbionts in *G. ruber* and seasonal variability in their photosynthetic activity act to modify the internal pH during calcification, by up to 0.2 units relative to ambient seawater.” This supports our line of argumentation above.

In another recent paper on B / Ca, Salmon et al. (2016) write: “We provide the first evidence for a strong positive relationship between area density (test thickness) and B / Ca, and reveal that this is consistent in all species studied, suggesting a likely role for calcification in controlling boron partitioning into foraminiferal calcite.” Their conclusion also supports our reasoning, that, mechanistically, increased photosynthesis may lead to higher calcification rates. Remarkably, Salmon et al. (2016) show that B / Ca of the non-symbiont-bearing species (*Globigerina bulloides* and *Globigerina inflata*) and even the symbiont-bearing species *G. sacculifer* are related to $[\text{CO}_3^{2-}]$ and $[\text{B}(\text{OH})_4^- / \text{HCO}_3^-]$. In our view, those results demonstrate the primary control by carbonate chemistry parameters not masked by symbiont photosynthesis. One could even argue that there is a positive trend for *O. universa* but that the natural range of $[\text{CO}_3^{2-}]$ variability (or borate/bicarbonate) is small (ca. $20 \mu\text{mol kg}^{-1}$ in the depth range 30 to 50 m) in comparison to the decoupling we carried out in controlled culture experiments. Interestingly, Henehan et al. (2016) propose a field calibration for *O. universa* that is very close to $\delta^{11}\text{B}$ of borate, suggesting that their “vital effects” are muted in the real ocean, especially the symbiont impact of raising the calibration curve above $\delta^{11}\text{B}$ of borate. This is supported by the observation of Hemleben and Bijma (1994) that *O. universa* occupies a subsurface maximum (in the Red Sea) between 20 and 60 m (Hemleben and Bijma, 1994, Fig. 5) and could explain why B / Ca in this species is not (completely) masked by symbiont photosynthesis (Salmon et al., 2016).

Our final conclusion is that, although controlled laboratory studies are the only means to clarify the mechanisms of proxy incorporation, field studies are required to determine to what extent vital effects determine species-specific offsets from the target parameters. For instance, the light level used in the culture experiments of Sanyal et al. (2001) was $\sim 380 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, providing a photon flux for maximum photosynthetic rates (P_{max}) of the symbionts (Spero and DeNiro, 1987; Spero and Parker, 1985; Spero and Williams, 1988). Consequently, the impact of photosynthesis on the *G. sacculifer* calibration of Sanyal et al. (2001) is fully

expressed. However, in the real ocean this species may experience lower irradiance, shifting the calibration curve more towards the borate values. In our study, the average irradiance in the culture jars was about $290 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, which is well below P_{max} of the symbionts and apparently closer to the irradiance conditions of their natural depth habitat. Therefore, the impact of photosynthesis is muted (Hönisch et al., 2003; Zeebe, 2003) and our laboratory calibration closer to the field calibration of (Henehan et al., 2016).

Laboratory experiments are usually carried out with foraminifera selected as model organisms for ease of availability and ability to be maintained in culture but, generally, state nothing about their suitability for paleo-studies. Field studies are much better to identify which species are best suited for down-core reconstructions. We agree with Henehan et al. (2015) that *G. ruber* is not a good choice for B / Ca as its primary relationship to carbonate chemistry parameters is, apparently, not very robust. However, other symbiont-bearing species, non-symbiotic planktonic foraminifera and deep-sea benthic foraminifera, may still be a viable option to use B / Ca for carbonate chemistry reconstructions.

5 Data availability

Data are included in the Supplement and archived on the Pan-gaea Database (Howes et al., 2016).

The Supplement related to this article is available online at doi:10.5194/bg-14-415-2017-supplement.

Competing interests. The authors declare that they have no conflict of interest.

Acknowledgements. Thanks are due to the sailors Jean-Yves Carval and Jean-Luc Prevost for their help and expertise with collection of foraminifera, to Samir Alliouane for assistance in the laboratory, Paul Mahacek for construction of the lighting equipment and Fabien Lombard for the use of his laboratory space and equipment. This work is a contribution to the European Union, Framework 7 Mediterranean Sea acidification under a changing climate project (MedSeA; grant agreement 265103).

Edited by: H. Kitazato

Reviewed by: two anonymous referees

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