Please find below the detailed response to the reviewers and the marked revised MS.

Anonymous Referee #1
Received and published: 20 June 2016

This study presents boron data (B/Ca and d11B) for planktonic foraminifera O. universa cultured in a decoupled carbonate system. The authors concluded that the B/Ca ratios correlate with [B(OH)4-/HCO3-], while the d11B values depend solely on pH. Although the data are fundamentally valuable, the culturing system is a concept recycled from a report by Kaczmarek et al. (2015b, BG), who used benthic foraminifera A. lessonii.
Moreover, the discussion is repetitive. In addition, the quality of the data and the discussion are inferior compared to that earlier report. If this work cannot venture beyond a case study, I think that it is difficult to justify its publication in BG.

We are a bit disappointed by the little justification provided by the reviewer to conclude that he or she thinks "that it is difficult to justify its publication in BG". We do agree that the concept used by Kaczmarek et al., 2015 (BG) is the same as the one used here. This was on purpose and makes perfect sense as the research comes from the same group and the aim was to have comparable experimental conditions for a benthic (Kaczmarek et al., 2015) and a planktonic species (this paper). Although, one could argue that there is maybe no *a priori* reason to expect that planktonic and benthic foraminifera behave differently, it remains to be investigated and documented. In addition, this is the first paper that investigates the boron isotope response in a planktonic species under experimentally decoupled carbonate chemistries (Allen and Hönisch (2012), who also decoupled the carbonate chemistry, only reported its impact on B/Ca). As such, laboratory experiments are the only way to develop a mechanic understanding of what the primary drivers of shell chemistry are. We therefore believe that this is a valuable addition to the scientific literature, especially for the growing "boron community".

Specific comments:
Methods: d11B data should be normalized to NIST SRM 951, not NIST SRM 610, as most of the cited reports of the literature do.

The reviewer is absolutely right that most of the cited reports in the literature normalize $d^{11}B$ to NIST SRM 951. This is due to the fact that almost all previously published papers on B/Ca and $d^{11}B$ use "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 measured using femto second laser ablation, for which we had to use a different (solid) standard (NIST SRM 610). As shown by several studies (Kasemann et al., 2001; le Roux et al., 2004; Fietzke et al., 2010), both standards are, within analytical uncertainty, isotopically equal. Hence, it doesn't make a difference if values are reported versus one or the other standard. It should be noted that fs laser ablation is matrix independent, i.e. it does not require a matrix matched standard and therefore allows us to use NIST SRM 610 for carbonates (also see the reply to reviewer 2).

We have changed the text as follows (line 320):
"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 measured using laser ablation, for which we used a different standard (NIST SRM 610). As shown by several studies (Kasemann et al., 2001; le Roux et al., 2004; Fietzke et al., 2010), both standards are, within analytical uncertainty, isotopically equal. Hence, for comparison between d^{11}B *O. universa* and d^{11}B of $B(OH)_4^-$ the isotopic difference between the two standards can be neglected and it does not make a difference if values are reported versus one or the other standard."

L345: Is d11B = 18.8‰ at pH 8.05 a mean value of the three experiments with different [CO3 2-] concentrations? Justify that calculation. This is also inconsistent with Fig. 3A.

We thank the reviewer for pointing this out. We have forgotten to change the numbers in the table. As the number of ablations per experimental condition is limited we have given preference to using median values. In a perfect Gausian distribution, the median has the same value as the mean. However, if the number of data points is low, the distribution may not be Gausian and then the median is less sensitive to "outliers".
The justification for plotting the mean of all $[CO_3^{2-}]$ experiments is that in the real ocean pH and $[CO_3^{2-}]$ co-vary. By combining d^{11}B for a range of $[CO_3^{2-}]$ we provide a number that can be used in modern surface waters having different $[CO_3^{2-}]$ (239 to 534µmol kg^{-1}). It should be noted that the median d^{11}B of the combined experiments at different $[CO_3^{2-}]$ is 18.8‰ but the same as for the intermediate $[CO_3^{2-}]$ at 285 µmol/kg. This $[CO_3^{2-}]$ at pH 8.05 is comparable to a $[CO_3^{2-}]$ of 296 µmol kg^{-1} at pH 7.9 and a $[CO_3^{2-}]$ of 258 µmol kg^{-1} at pH 7.7.

We have corrected the inconsistency with Fig. 3A.

L347-349: In spite of the large analytical error, it is difficult to conclude that no significant effect of [CO3 2-] on d11B was found.

We agree with the reviewer, that there is an effect of $[CO_3^{2-}]$ on d^{11}B and this is now discussed in the text. However, in reality this effect will be minor as in the real ocean pH and $[CO_3^{2-}]$ co-vary and the range of $[CO_3^{2-}]$ is much more limited than in our experimental treatments).

We have added a paragraph to the result section: "δ^{11}B increases with increasing $[CO_3^{2-}]$ at constant pH_T from 17.2‰ at 238 µmol/kg CO_3^{2-} to 19.9‰ at 534 µmol/kg 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 difference between the mean δ^{11}B values of the $[CO_3^{2-}]$ treatments 239 and 286 µmol/kg were close to significance but only between 239 and 534 µmol/kg the difference was significant (Supplement Table 3). Because, this range in $[CO_3^{2-}]$ is beyond that of the real ocean and because pH and $[CO_3^{2-}]$ co-vary, we believe that this observation is only important for a better understanding of the δ^{11}B controls and does not significantly impact existing calibrations."

Is there any correlation between B/Ca and d11B?

As expected, based on first principles, there is a correlation between B/Ca and $d^{11}B$. We have added 2 plots in the supplement file and a short paragraph in the results section:" Based on first principles, we predict a positive correlation between B/Ca and $d^{11}B$ because at higher pH not only the isotopic composition of borate gets heavier but its concentration also increases. Figures 2A (supplement) shows the individual B/Ca, $d^{11}B$ pairs per treatment. As expected for individual LA shell analyses is the inter specimen variability quite large. Individual B/Ca ratios vary by almost 50% in each treatment and individual $d^{11}B$ 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 $d^{11}B$ in some of the treatments, we believe that the individual B/Ca, $d^{11}B$ pairs within a treatment demonstrate are However, the average values for the four treatments with [CO_3^{-2}] between 238 – 297 µmol kg^{-1} do show a positive correlation between B/Ca and $d^{11}B$. The "outlier" (treatment at pH 8.05; [CO_3^{-2}] = 534) can be explained by the, relative to the other pH = 8.05 treatments, high [HCO_3^{-}].".

L447-448: The meaning of "The proxy should therefore be ground-truthed using core top samples" is unclear.

Even if experimental data provide insight into fractionation mechanisms, field verification (both water column and sediment) is needed to investigate how seasonality, population dynamics and other environmental factors, not tested under controlled laboratory conditions, affect their geochemistry and to which extend. It also allows verification of which species are suited best for paleo reconstructions (*O. universa* is just our lab rat). This issue is extensively discussed in the reply to Michael Henehan.

References: The cited Hanehan et al. (2015) is missing from the list of references.

We thank the reviewer for point this out. The reference has been added.

Tables 1 & 3: Order the data at pH 8.05 in ascending order according to [CO3 2-].

Very good point. Done!

Fig. 3: Put alphabet characters on each graph. The median values are shown as red circles, not black.

We have added the alphabet characters and changed black to red.

Anonymous Referee #2
Received and published: 3 June 2016

"general comments"

The submitted discussion paper provides a boron data set (d11B and B/Ca) of foraminifera from a culturing study performed modifying pH and [CO3 2-] in a decoupled way. It relates to an interesting topic: proxy calibration for paleoreconstruction of key parameters of the marine carbonate system. While the efforts involved in culturing are truely acknowledged the extend of data and its discussion unfortunately are not great. As it stands I would consider this manuscript as a data brief and I am not convinced it will make an exciting contribution to BG.

Again, we are a bit disappointed by the little justification provided by the reviewer to conclude that this manuscript will not make "an exciting contribution to BG". As argued above in our reply to the first reviewer, this is the first paper that reports the boron isotope response in a planktonic species under experimentally decoupled carbonate chemistries (Allen and Hönisch (2012), who also decoupled the carbonate chemistry, only reported its impact on B/Ca). As such, laboratory experiments are the only way to develop a mechanic understanding of what the primary drivers of shell chemistry are. We therefore believe that this is a valuable addition to the scientific literature, especially for the growing "boron community".

"specific comments"

Most of the method section's content can be found in the cited literature. Thus, it get's too much space in the manuscript and could be moved into a dedicated part of the supplements. The normalization procedures for d11B read confusing. I assume d11B of the culturing water has been measured, as the data are provided in table 1. I do miss information on how these data have been generated. I would assume using MC-ICPMS, relative to NBS SRM-951?

We think that the method section is important. Where possible we refer to the literature. The first author is a native English speaking person and read through the section on normalization procedures again and made the appropriate changes. We have also added a section on the boron analyses of the culture water:

"***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 ($^{11}B/^{10}B = 4.04362 \pm 0.00137$) (Catanzaro, 1970) where:

$$\delta^{11}B_{sample}(‰) = \left[\frac{\left({}^{11/10}B\right)_{sample}}{\left({}^{11/10}B\right)_{NISTSRM\ 951a}} - 1\right] \times 1000$$

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 ^{11}B measurements of seawater (from the Atlantic Ocean) and secondary boron standards (AE 120, 121, 122) with published (accepted) results. Our estimate of ^{11}B$_{SW}$ of 39.80.4‰ (2s, n = 30) are independent of sample size and are in agreement with published values of 39.6 ± 0.4‰ (Foster et al., 2010) and 39.7 ± 0.6‰ (Spivack and Edmond, 1987). Moreover, our ^{11}B estimates of SRM AE-120 (-20.2 ± 0.5 ‰, 2s, n = 33), SRM AE-121 (19.8 ± 0.4 ‰, 2s, n =16), SRM AE-122 (39.6 ± 0.5 ‰, 2s, 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.(2015)."

The water composition is massively modified (10xB concentration and about 35 permill lighter than natural seawater). That's fine, the normalization should allow for comparability of the data. So, water data are presumably expressed relative to NBS951 (conventional delta11B notation). LA-MC-ICPMS data relate to NIST SRM-610. Nothing is mentioned about any further normalization, regarding differences between both SRMs. It appears the authors assume both to have identical boron isotopic composition.

That is correct. It has been shown by several studies (Kasemann et al., 2001; le Roux et al., 2004; Fietzke et al., 2010) that both standards are isotopically the same within analytical uncertainty.

When using a standard of an entirely different matrix during the laser analysis of foraminifera (silicate vs. carbonate), and ablating quite different amounts of both, some justification is needed to convince readers that no offsets (analytical artefacts) compromise the data.

Femto second laser ablation is matrix independent, i.e. it does not require a matrix matched standard and therefore allows us to use NIST SRM 610 (a glass) for carbonates. The fs laser ablation process is fundamentally different from ns laser ablation used by most people. When the pulse length is shorter than 10 ps (Hergenröder et al., 2006) the laser energy can be deposited into the material before it can thermally equilibrate. Femtosecond ablation also provide smaller aerosol particle sizes. The matrix independency of fs laser ablation has been demonstrated by many papers (some of which published in journals that are usually read by geochemists and paleoceanographers) (e.g. Chmeleff et al., 2008; Horn et al., 2006; Oeser et al., 2014; Schuessler and von Blanckenburg, 2014; Kaczmarek et al., 2015; Lazarov et al., 2015; Lazarov and Horn, 2015) and therefore allows us to use NIST SRM 610 for the carbonates.

As the boron concentrations can differ between samples 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 d^{11}B of the standard.

References

Chmeleff, J., Horn, I., Steinhoefel, G. and von Blanckenburg, F. (2008) In situ determination of precise stable Si isotope ratios by UV-femtosecond laser ablation high-resolution multi-collector ICP-MS. Chemical Geology 249, 155-166.

Fietzke, J., Heinemann, A., Taubner, I., Bohm, F., Erez, J., and Eisenhauer, A.: Boron isotope ratio determination in carbonates via LA-MC-ICP-MS using soda-lime glass standards as reference material, J. Anal. Atom. Spectrom., 25, 1953–1957, 2010.

Horn, I., von Blanckenburg, F., Schoenberg, R., Steinhoefel, G. and Markl, G. (2006) In situ iron isotope ratio determination using UV-femtosecond laser ablation with application to hydrothermal ore formation processes. Geochimica et Cosmochimica Acta 70, 3677-3688.

Kasemann, S., Meixner, A., Rocholl, A., Vennemann, T., Rosner, M., Schmitt, A. K., and Wiedenbeck, M.: Boron and Oxygen Isotope Composition of Certified Reference Materials NIST SRM 610/612 and Reference Materials JB-2 and JR-2, Geostandard.Newslett., 25, 405–416, 2001

Kaczmarek, K., Horn, I., Nehrke, G. and Bijma, J. (2015) Simultaneous determination of δ11B and B/Ca ratio in marine biogenic carbonates at nanogram level. Chemical Geology 392, 32-42.

Lazarov, M. and Horn, I. (2015) Matrix and energy effects during in-situ determination of Cu isotope ratios by ultraviolet-femtosecond laser ablation multicollector inductively coupled plasma mass spectrometry. Spectrochimica Acta Part B: Atomic Spectroscopy 111, 64-73.

Le Roux, P. J., Shirey, S. B., Benton, L., Hauri, E. H., and Mock, T. D.: In situ, multiple-multiplier, laser ablation ICP-MS measurement of boron isotopic composition (δ11B) at the nanogram level, Chem. Geol., 203, 123–138, 2004.

Oeser, M., Weyer, S., Horn, I. and Schuth, S. (2014) High-Precision Fe and Mg Isotope Ratios of Silicate Reference Glasses Determined In Situ by Femtosecond LA-MC-ICP-MS and by Solution Nebulisation MC-ICP-MS. Geostandards and Geoanalytical Research 38, 311-328.

Schuessler, J.A. and von Blanckenburg, F. (2014) Testing the limits of micro-scale analyses of Si stable isotopes by femtosecond laser ablation multicollector inductively coupled plasma mass spectrometry with application to rock weathering. Spectrochimica Acta Part B: Atomic Spectroscopy 98, 1-18.

Steinhoefel, G., Horn, I. and von Blanckenburg, F. (2009) Matrix-independent Fe isotope ratio determination in silicates using UV femtosecond laser ablation. Chemical Geology 268, 67-73.

We have added the following text to the method section:

"It should be noted that the fs laser ablation process is fundamentally different from ns laser ablation. When the pulse length is shorter than 10 ps (Hergenröder and Hommes, 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 is matrix independent (e.g. Chmeleff et al., 2008; Horn et al., 2006; Oeser et al., 2014; Schuessler and von Blanckenburg, 2014; Kaczmarek et al., 2015; Lazarov et al., 2015; Lazarov and Horn, 2015), i.e. it does not require a matrix matched standard and therefore allows us to use NIST SRM 610 (a glass) as a reference for carbonates.
As the boron concentrations is different 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 d^{11}B of the standard.

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 measured using laser ablation, for which we used a different standard (NIST SRM 610). As shown by several studies (Kasemann et al., 2001; le Roux et al., 2004; Fietzke et al., 2010), both standards are, within analytical uncertainty, isotopically equal. Hence, it doesn't make a difference if values are reported versus one or the other standard."

Have the foraminifera shells been treated chemically prior to laser analyses (e.g.oxydative cleaning)?

Text added lines 226-228:
Prior to analysis, specimens were harvested, bleached in NaOCl (active chlorine: 4.6 %) for 6 hours, rinsed four times using de-ionized water, and dried for 12 h at 50 °C.

Nothing is mentioned about the quite large variability within the d11B data of foraminifera within each treatment group. How do you explain this observation?

We like to draw the reviewer's attention to studies about single foram analyses such as that carried out by Rollin-Bard & Erez (2009) using a nanoSIMS and demonstrating a d^{11}B variability between 4.7 to 12.2 per mill. For laser ablation
Kaczmarek et al. (2015a,b) demonstrated that intra specimen d^{11}B variability was typically around 5 to 6 permil. The spread shown in our data is due to both inter- and intra-shell variability. Branson et al. (2015) have shown that boron is not homogeneously distributed in foraminiferal shells. In fact, the boron concentration (and hence B/CA) shows distinct high and low concentration boron bands which are probably diachronous with the well-known high and low Mg banding. Recently, Sadekov and his Cambridge colleagues showed a significant ontogenetic variability in boron concentration (B/Ca) and that the d^{11}B variability across these bands in the shell wall is about 10 permil (poster at ICP12, Utrecht, 2016)! However, they also showed that the mean d^{11}B value was close to the d^{11}B value from wet chemical analysis.

Besides the inter-shell variability, there is also significant variability between individual specimens. De Nooijer et al. (2014), showed that, even for genetically identical specimens (clones) grown under identical conditions, their geochemical signatures can be significantly different. Differences in Sr/Ca and Mg/Ca between clone groups were similar to the intra-clone group variability, suggesting that any genetic differences between the clone groups did not affect trace element partitioning. Instead, variability in Mg/Ca appears to be inherent to the process of bio-calcification itself. There is no *a priori* reason to assume that this would be fundamentally different for B/Ca or d^{11}B for that matter.

When applying wet chemical boron isotope analyses, as most other groups do, many foraminiferal shells are combined for single, duplicate or triplicate measurements of the same sample. Hence, intra- and inter-specimen variability is then averaged and the remaining variability within the data is related to analytical issues only (and unrelated to biological/physiological and/or population dynamical impacts).

References

Branson, O., Kaczmarek, K., Redfern, S.A.T., Misra, S., Langer, G., Tyliszczak, T., Bijma, J. and Elderfield, H. (2015) The coordination and distribution of B in foraminiferal calcite. Earth and Planetary Science Letters 416, 67-72.

Kaczmarek, K., Langer, G., Nehrke, G., Horn, I., Misra, S., Janse, M. and Bijma, J. (2015) Boron incorporation in the foraminifer Amphistegina lessonii under a decoupled carbonate chemistry. Biogeosciences 12, 1753-1763.

Kaczmarek, K., Horn, I., Nehrke, G. and Bijma, J. (2015) Simultaneous determination of δ11B and B/Ca ratio in marine biogenic carbonates at nanogram level. Chemical Geology 392, 32-42.

de Nooijer, L.J., Hathorne, E.C., Reichart, G.J., Langer, G. and Bijma, J. (2014) Variability in calcitic Mg/Ca and Sr/Ca ratios in clones of the benthic foraminifer Ammonia tepida. Marine Micropaleontology 107, 32-43.

And considering this variability I am somewhat surprised the data (figure 3A) do match the inorganic borate curve (Klochko-curve) more or less perfectly. Statistically this is almost impossible, considering the mean 2SE to be >1.5permill.

Obviously, we were very surprised ourselves and checked our calculations several times. If we wanted to cheat we would probably not have come up with a more or less perfect match. Wouldn’t that be too obvious? However, at the end we are glad that the reviewer raised this issue as we went through the calculations again and again and did discover a silly mistake in the spreadsheet that was overlooked before and affected the d^{11}B value at pH 8.05. This has now been corrected.

Reply to the “interactive comment” by Michael Henehan.

First of all, we like to thank Michael for his extensive, critical and well justified comments.

1) "I have some concerns about the spread in the data and the calculation and propagation of uncertainties, and would suggest the authors at some point explicitly mention that the uncertainty poses limitations for how much can be interpreted from these data".

In contrast to “wet chemistry” does Laser ablation record the inhomogeneous B distribution (“boron banding” see Branson et al., 2015 EPSL) and individual shell analysis captures intra-specimen differences (see reply to reviewer 2). As impressively demonstrated by Sadekov et al. (2016) is the variability in Both B/Ca and d^{11}B recurring in each chamber and therefore represents real data of high quality. This is supported by the fact that the value of average laser data are very close to wet chemical analyses were multiple specimens are dissolved and the intra- and inter-variability is “averaged” before the analysis. Therefore, we disagee that the “uncertainty poses limitations for how much can be interpreted from these data”.

We have added the following text in the method section: “Laser ablation, in contrast to “wet chemical” analysis, records the inhomogeneous boron distribution (“boron banding” see Branson et al., 2015) within a specimen and individual shell analysis captures inter-specimen differences. As impressively demonstrated by Sadekov et al. (2016) is the variability in both, B/Ca and d^{11}B recurring 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 were multiple specimens are dissolved and the intra- and inter-variability is “averaged” before the analysis. The intra-specimen d^{11}B variability in *Cibicidoides wuellerstorfi* is up to ca. 10 permil (Sadekov et al. (2016), while the inter-specimen δ^{11}B variability of *Amphistegina lessonii* from the same treatment is ca. 6 permil (Kaczmarek et al., 2015b). Histograms of single-foram δ^{11}B measurements from each of our pH treatments (supplementary Fig. 1) show that the laser ablation data is normally distributed (p-values from Shapiro-Wilk-tests are all higher than 0.05). This is confirmed by the box plots were the average and median values are very close to each other. Therefore, the relatively large standard errors of laser ablation analyses do not present a limitation for how much can be interpreted from the data.”

References:

Sadekov, A., Kerr, J., Langer, G., de la Fuente, M., Skinner, L. and Elderfield, H. 2016. Understanding the mechanisms behind boron elemental and isotopic fractionation in the benthic foraminifera Cibicidoides wuellerstorfi. Poster, ICP12, Utrecht.

Branson, O., Kaczmarek, K., Redfern, S.A.T., Misra, S., Langer, G., Tyliszczak, T., Bijma, J. and Elderfield, H. (2015) The coordination and distribution of B in foraminiferal calcite. Earth and Planetary Science Letters 416, 67-72.

2) "Including some measurements of widely analysed calcium carbonate standard material (such as JCP or JCT) would be really helpful in clearly demonstrating the efficacy of the technique".

JCP and JCT are biological samples and probably have variable B/Ca and $d^{11}B$ even at the micro-scale (they are powders). The laser spot will be too large to analyze individual grains and we have no means to analyze powders with laser ablation (yet). We agree that it would be very valuable to demonstrate how (the average of) LA data relate to wet chemistry analyses of both standards. This is something that we plan to do in the near future and has our first priority as soon as we have our own laser set-up properly. For the BG msc we will refer to papers demonstrating matrix independency (see our response to reviewer 2) and that we can use glass standards to relate to carbonate samples and standards.

We have added some text to the method section:

“It should be noted that the fs laser ablation process is fundamentally different from ns laser ablation. When the pulse length is shorter than 10 ps (Hergenröder and Hommes, 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 is matrix independent (e.g. Chmeleff et al., 2008; Horn et al., 2006; Oeser et al., 2014; Schuessler and von Blanckenburg, 2014; Kaczmarek et al., 2015; Lazarov et al., 2015; Lazarov and Horn, 2015), i.e. it does not require a matrix matched standard and therefore allows us to use NIST SRM 610 (a glass) as a reference for carbonates.

As the boron concentrations are different 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 $d^{11}B$ of the standard.

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 measured using laser ablation, for which we used a different standard (referenced against NIST SRM 610). As shown by several studies (Kasemann et al., 2001; le Roux et al., 2004; Fietzke et al., 2010), both standards are, within analytical uncertainty, isotopically equal. Hence, for comparison between $\delta^{11}B$ *O. universa* and $\delta^{11}B$ of $B(OH)_4^-$ the isotopic difference between the two standards can be neglected and it does not make a difference if values are reported versus one or the other standard. ”

3) You suggest "inclusion of measurements of open ocean O. universa".

You are absolutely right again that we could have done that. However, this paper focusses on the controls of boron incorporation only and we have chosen to leave the comparison of field and laboratory grown specimen as well as the impact of increased boron concentrations in culture water for a separate paper. In the past we have increased the boron concentration in culture water for obvious practical reasons. Hönisch et al. (2003) and Zeebe et al. (2003) demonstrate that it should not have an impact on $d^{11}B$ but we would like to investigate that a bit further.

Hönisch, B., Bijma, J., Russell, A.D., Spero, H.J., Palmer, M.R., Zeebe, R.E. and Eisenhauer, A. (2003) The influence of symbiont photosynthesis on the boron isotopic composition of foraminifera shells. Marine Micropaleontology 49, 87-96.

Zeebe, R.E., Wolf-Gladrow, D.A., Bijma, J. and Honisch, B. (2003) Vital effects in foraminifera do not compromise the use of delta B-11 as a paleo-pH indicator: Evidence from modeling - art. no. 1043. Paleoceanography 18, 1043-1043.

4) "…….inclusion of measurements of open ocean O. universa……would test the hypothesis put forward for the apparently muted vital effects." …… " It would also address the issue of bicarbonate control- since there are a number of studies that show that just because these patterns can be seen in culture, it doesn't mean they will hold up outside of the lab."

Analysis of open ocean *O. universa* can only partly address the point you make. In the lab we can perfectly control the environment the forams "see" and we can decouple parameters and extend each of them individually beyond the natural range while keeping the rest constant. Although, there is no *a priori* reason to assume that they would respond differently in the lab than in the field, this is difficult to prove. In the field there are many variables that are not constant, parameters cannot be decoupled and usually the ranges are limited. There may be ontogenetic migration, etc. I think that your recent paper in EPSL is a nice example and we will refer to it as Henehan et al., (in review) if it is not out yet. Your suggestion that the impact of photosynthesis on d^{11}B of *O. universa* (or shell geochemistry in general) as observed in the lab may be muted in the field is a valid point and in line with my observations on their population dynamics (Hemleben et al., 1994): "*Orbulina universa* d'Orbigny shows a subsurface maximum" and our data in the Red Sea indicate a depth habitat between 20-60 meters (Fig. 5).

We have added some text to clarify this issue: "Interestingly, Henehan et al. (2016) propose a field calibration for *O. universa* that is very close to d^{11}B of borate, suggesting that their "vital effects" are muted in the real ocean, especially the symbiont impact of raising the calibration curve above d^{11}B of borate. This is supported by the observation of Hemleben et al., (1994) that *O .universa* occupies a subsurface maximum (in the Red Sea) between 20-60 meters (Hemleben et al., 1994; Fig. 5) and could explain why B/Ca in this species is not (completely) masked by symbiont photosynthesis (Salmon et al., 2016)."

Hemleben, C. and Bijma, J. (1994) Foraminiferal population dynamics and stable carbon isotopes., in: Zahn, R., Pedersen, T.F., Kaminski, M., Labeyrie, L. (Eds.), Carbon Cycling in the Glacial Ocean: Constraints on the Ocean´s Role in Global Change. Elsevier, Fellhorst, pp. 145-166.

5) you suggest that "In the face of the data from Babila et al., Henehan et al., Salmon et al. (2016, EPSL) and the excellent review by Allen and Hönisch (2012), the authors should remove suggestions of using B/Ca as a proxy for the second carbonate system parameter, unless they can show evidence to support this relationship standing up out of the culture lab."

You are right that it will probably be impossible for *G. ruber* (the most "autotrophic" of all symbiont bearing foraminifera) to use B/Ca downcore, but for non-symbiotic planktonic forams or benthics, it may still be a viable option. Below we will explain why we think that the relationship between PO4 and B/Ca that you describe for *G. ruber* in the G3, 2015 paper may be a red herring (but we cannot prove it because it would require new culture experiments using micro-electrodes).

First of all, we fully agree that we were not clear at all regarding our comment related to a possible correlation between PO4 and B/Ca as suggested by Henehan et al. (2015). We wrote: "....we believe that this relationship results from a co-variation between ocean carbonate chemistry and nutrients because respiration of organic matter will release both carbon and nutrients." We were not referring to bulk ocean conditions but to their ambient environment. Forams, and especially symbiont bearing planktonic forams **never** "see" the bulk ocean carbonate chemistry (see e.g micro-electrode studies by Rink et al. (1998) and the modelling study by Wolf-Gladrow et al. (1999)). They only see their ambient carbonate chemistry as modulated by their own life processes as well as by symbiont photosynthesis and respiration (therefore our calibrations are empirical and not mechanistic). Surface dwelling deep sea benthics probably come closest to recording real bulk carbonate chemistry conditions, although the nutrient and carbonate chemistry gradients in the fluffy layer (which is the time when they grow and reproduce, I assume) may be very strong.

In section 2.3 of your G3 paper you describe how you produce the environmental data (nutrients and carbonate chemistry). This is indeed the best you can do, but you will agree that the average (**annual**) **estimates** you get from extrapolation from grid point, etc... may not reflect real conditions during growth of the forams. In general, we would expect a positive linear relationship between nutrients and DIC and a negative one between nutrients and pH. When nutrients (PO_4) are high, DIC is usually high and pH is low (normal deep water conditions or classical upwelling but also temperate ocean after winter mixing). High nutrients lead to higher primary productivity, consuming nutrients and DIC and increasing pH (normal sfc ocean conditions during spring bloom). The bottom line, in our view, is that the absence of the above mentioned relationships question the consistency between analysed B/Ca and the estimated environmental data.

If we then turn to the data used for the cultures and the plankton tows in the Gulf of Eilat, those are of a very different quality as everything has been analysed/determined when the forams where actually adding carbonate.

For the following it is important to realise that, of all symbiont bearing planktonic forams, *G. ruber* is the most "autotropic". Your fig. 4 demonstrates the close relationship between carbonate chemistry and B/Ca (when PO_4 constant!). Fig 7. shows a clear correlation between bulk PO_4 and the loss of a correlation with bulk carbonate chemistry (pH). However, we are convinced that if you would measure ambient pH (or $[CO_3^{2-}]$) at elevated PO_4, both (pH and $[CO_3^{2-}]$) would be significantly higher! Hence, even if the correlation with bulk pH is lost, the correlation with ambient pH (or $[CO_3^{2-}]$, $[HCO_3^{-}]$) will probably still be there. We cannot prove it to you but micro-electrode measurements could! In addition, there is no obvious direct link between PO_4 and B/Ca. Mechanistically, probably via increased photosynthesis leading to higher calcification rates. We show (Kaczmarek et al., 2015b) that boron partitioning increases with increasing growth rate in inorganic precipitation experiments.

Early work by the pioneers of foram biology and calcification (Bé, Anderson, Hemleben, Spindler, Erez, Spero, Caron, etc.) has clearly demonstrated the huge impact of symbionts on foram shell growth, e.g.:

Bé, A.W.H. (1965) The influence of depth on shell growth in globigerinoides sacculifer (brady). Micropaleontology 11, 81-97.)

Bé, A.W.H., Spero, H.J. and Anderson, O.R. (1982) Effects of symbiont elimination and reinfection on the life processes of the planktonic foraminifer globigerinoides sacculifer. Marine biol 70, 73-86.

Caron, D.A., Bé, A.W.H. and Anderson, O.R. (1981) Effects of variations in light intensity on life processes of the planktonic foraminifer globigerinoides sacculifer in laboratory culture. J. Mar. Biol. Assoc. U.k 62, 435-452.

Spero, H.J. and Parker, S.L. (1985) Photosynthesis in the symbiotic planktonic foraminifer Orbulina universa, and its potential contribution to oceanic primary productivity. Journal of Foraminiferal Research 15, 273-281.

Jørgensen, B.B., Erez, J., Revsbech, N.P. and Cohen, Y. (1985) Symbiotic photosynthesis in a planktonic foraminiferan, Globigerinoides sacculifer (Brady), studied with microelectrodes. Limnology and Oceanography 30, 1253-1267.

Hemleben, C., Spindler, M., Breitinger, I. and Ott, R. (1987) Morphological and physiological responses of Globigerinoides sacculifer (Brady) under varying laboratory conditions. Marine Micropaleontology 12, 305-324.

I hope this explains our point a bit better. PO_4 will increase symbiont photosynthesis, which raises ambient pH (and $[CO_3^{2-}]$) and effectively decouples it from the bulk ocean carbonate chemistry.

other references:

Wolf-Gladrow, D.A., Bijma, J. and Zeebe, R.E. (1999) Model simulation of the carbonate system in the microenvironment of symbiont bearing foraminifera. Marine Chemistry 64, 181-198.

Babila et al. (2014) write: "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 ~0.2units relative to ambient seawater." This is again about *G. ruber* and supports the arguments we suggested above.

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." This conclusion also supports our line of argumention above, that "Mechanistically, probably via increased photosynthesis leading to higher calcification rates." The subtropical gyre has negligible dissolved phosphate, hence they could not explicitly test your B/Ca relationship with [PO_4], but we bet they would have found it. Besides *G. ruber* they also looked at *orbulina* and *truncatulinoides*.

The sediment trap is at 1500m and calcification depths are calculated using $d^{18}O$ and Mg/Ca. This is the best they can do but also means that there is no real control on depth habitat. As the waters around the site are oligotrophic, you can expect that symbionts may overprint the primary relationship between B/Ca and carbonate chemistry parameters (as Babila et al. (2014) and you (Henehan et al., 2015) demonstrated for *G.ruber*). If available, it would be great to correlate B/Ca with monthly/seasonal light attenuation data for that site.

Their fig 3 shows, first of all, that *G. ruber, O. universa* and and *G. trunc* are all over the place but that the non-symbiont species (*G. bulloides* and **G. inflata**) and even *G. sacculifer* seems to show a trend with the three carbonate parameters (our guess showing a primary control by carbonate chemistry parameters). We would even argue that there is still a positive trend for *O. universa* (but for borate/bicarbonate the range is too small and the system in the field it is not decoupled). Second, you will agree that T and carbonate chemistry parameters at this site are correlated. Eventhough, *O. universa* is also a symbiont "battlestar", it is noteworthy that Salmon et al. conclude that "Higher B/Ca values significantly correlate with larger tests but only in *G. ruber*" (as it is the most autotrophic of all symbiont bearing species and larger specimens harbor more symbionts).

The bottom line is that field studies are not suitable for elucidating the mechanisms but are **VERY** helpful in showing which species are not good for paleo reconstructions of the carbonate system, and we agree with you that *G. ruber* is one of them.

We have substantially changed our discussion of using B/Ca as a proxy for the second carbonate system parameter and do justice to the fact that the above mentioned studies show a decoupling of the primary relationship.

We have added the following text to the last part of the discussion: “Recently, Henehan et al., (2015) showed that B/Ca in *G. ruber* collected with a plankton net was perfectly correlated to [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 e.g. $B(OH)_4^-$]/[HCO_3^-]. Based on plankton tow, sediment trap and core-top data, they concluded that, apparently, B/Ca in *G. ruber* is controlled by [PO_4^{3-}]. However, it should be noted that foraminifera, and especially symbiont bearing planktonic foraminifers never “see” the bulk ocean carbonate chemistry (e.g. e.g micro-electrode study by Rink et al. (1998) and the modelling study by Wolf-Gladrow et al. (1999)). They only “see” their ambient carbonate chemistry as modulated by their own life processes and symbiont photosynthesis and respiration (so called “vital effects”). Existing calibrations and field relationships are therefore purely empirical and not mechanistic.

Water masses usually show a covariation between nutrients and carbonate chemistry driven by community photosynthesis and respiration. When nutrients are high, DIC is usually high and pH is low and *vice versa*. However, the ambient carbonate chemistry of the foraminifer and the bulk seawater chemistry can be decoupled. We note that, of all symbiont bearing planktonic Foraminifera, *G. ruber* is probably the most "autotropic" (Bijma et al., 1992). Although we cannot prove it, we assume that symbiont photosynthetic rates are higher at elevated $[PO_4^{3-}]$ (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 $[CO_3^{2-}]$, $[HCO_3^-]$) may still hold up. At this point, there is no obvious direct link between $[PO_4^{3-}]$ and B/Ca and we believe that, mechanistically, it can be explained by increased photosynthesis and/or higher calcification rates. Kaczmarek et al., 2015b) 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, 1965; Bé et al., 1982; Caron et al., 1981; Spero and Parker, 1985; Jørgensen et al., 1985; Hemleben et al., 1987) has clearly demonstrated the huge impact of symbionts on foraminiferal shell growth. Interestingly, Babila et al. (2014) write: "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 species (*G. bulloides* and *G. inflata*) and even the symbiont bearing species *G. sacculifer* are related to $[CO_3^{2-}]$ and $[B(OH)_4^-/HCO_3^-]$, showing a 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 for borate/bicarbonate is small in comparison to the decoupling we carried out in controlled culture experiments.

Our final conclusion is that field studies are not suitable for elucidating the mechanisms of proxy incorporation but are very helpful in showing which species are the golden standard for paleo reconstructions of the carbonate system, and we agree with Henehan et al. (2015) that *G. ruber* is not a good choice as its primary relationship to carbonate chemistry parameters is not very robust. However, other symbiont bearing species, non-symbiotic planktonic foraminifera and deep sea benthics, may still be a viable option to use B/Ca for carbonate chemistry reconstructions."

References:

Rink, S., Kühl, M., Bijma, J. and Spero, H.J. (1998) Microsensor studies of photosynthesis and respiration in the symbiotic foraminifer *Orbulina universa*. Marine Biology 131, 583-595.

Wolf-Gladrow, D.A., Bijma, J. and Zeebe, R.E. (1999) Model simulation of the carbonate system in the microenvironment of symbiont bearing foraminifera. Marine Chemistry 64, 181-198.

Bijma, J., Hemleben, C., Oberhänsli, H. and Spindler, M. (1992) The effects of increased water fertility on tropical spinose planktonic foraminifers in laboratory cultures. Journal of foraminiferal research 22, 242-256.

Bé, A.W.H. (1965) The influence of depth on shell growth in globigerinoides sacculifer (brady). Micropaleontology 11, 81-97.

Bé, A.W.H., Spero, H.J. and Anderson, O.R. (1982) Effects of symbiont elimination and reinfection on the life processes of the planktonic foraminifer globigerinoides sacculifer. Marine biol 70, 73-86.

Caron, D.A., Bé, A.W.H. and Anderson, O.R. (1981) Effects of variations in light intensity on life processes of the planktonic foraminifer globigerinoides sacculifer in laboratory culture. J. Mar. Biol. Assoc. U.k 62, 435-452.

Spero, H.J. and Parker, S.L. (1985) Photosynthesis in the symbiotic planktonic foraminifer Orbulina universa, and its potential contribution to oceanic primary productivity. Journal of Foraminiferal Research 15, 273-281.

Jørgensen, B.B., Erez, J., Revsbech, N.P. and Cohen, Y. (1985) Symbiotic photosynthesis in a planktonic foraminiferan, Globigerinoides sacculifer (Brady), studied with microelectrodes. Limnology and Oceanography 30, 1253-1267.

Hemleben, C., Spindler, M., Breitinger, I. and Ott, R. (1987) Morphological and physiological responses of Globigerinoides sacculifer (Brady) under varying laboratory conditions. Marine Micropaleontology 12, 305-324.

Babila, T.L., Rosenthal, Y. and Conte, M.H. (2014) Evaluation of the biogeochemical controls on B/Ca of Globigerinoides ruber white from the Oceanic Flux Program, Bermuda. Earth and Planetary Science Letters 404, 67-76.

Salmon, K.H., Anand, P., Sexton, P.F. and Conte, M. (2016) Calcification and growth processes in planktonic foraminifera complicate the use of B/Ca and U/Ca as carbonate chemistry proxies. Earth and Planetary Science Letters 449, 372-381.

6) In the next paragraph you say: "More generally, there are a few instances in the intro and discussion where preference is given to detailing the findings of older, and since superceded studies, rather than cutting straight to the new data coming out of the community and dealing with the questions they raise."

We fully agree, this is our mistake and due to the fact that this msc has been around for too long and we didn't properly update.

We have added and discussed the newest literature.

"Specific points"

Lines 25-27: the sentence here does not make the necessary link between borate substituting into carbonate and why this would then make it a carbonate ion proxy. Need to point out in a sentence like this that borate is more abundant at higher pHs.

Has been changed to read: "As $B(OH)_4^-$ is substituted into the biogenic calcite lattice in place of CO_3^{2-} and both borate and carbonate ion are more abundant at higher pHs it has been suggested early on that B/Ca ratios in biogenic calcite are a possible proxy for $[CO_3^{2-}]$."

Line 37-40 (and throughout): I have some serious concerns over the point being made here- that B/Ca is a useful second carbonate system parameter. Many experiments have shown (including ours- Henehan et al. 2015, G3) that you can produce a pH dependent shift in B/Ca in culture experiments where you change only the carbonate system. However, in the open ocean these relationships often fall down, because there are other controls on boron incorporation- see for example my paper, or excellent papers by Babila et al, or Allen and Hönisch (2012) or a really great paper just out by Kate Salmon et al. in EPSL. This abstract, and indeed the paper, is strongly advocating the use of B/Ca to derive the whole carbonate system, but this is based only on culture experiments and ignores the evidence in other papers that shows that really B/Ca is not at all reliable in open ocean foraminifera as a tracer of the carbonate system. These open ocean studies must be considered and the claims on behalf of B/Ca as a proxy needs to be removed.

Please see our reply to 5) above. We argue that there is possibly still a primary relationship between the boron uptake and $[B(OH)_4^-]/[HCO_3^-]$ as shown in our culture study. At this stage we cannot prove this but microelectrode studies could (e.g. Rink et al., 1998). We further agree with you, that in the field, the primary relationship between B/Ca and $[B(OH)_4^-]/[HCO_3^-]$ can be completely masked by other parameters and that therefore B/Ca loses its potential as a robust proxy. We have discussed this now in the last part of the revised msc under "Proxy implications".

Line 68: Line beginning 'At low'.. This sentence would be better off earlier where you mention pH-dependent speciation in Line 65. As it stands the thread of the paragraph is a little disjointed.

Done as suggested

Line 79: Suggest removing pteropods from here since people don't tend to use them for boron work.

Done as suggested

Lines 81-85: Pioneering as these studies were, the field has moved on quite a lot since then, and I am not sure I see the logic in dwelling so long on the specific findings of these studies when they have been superceded by better estimates of these pH values. Indeed, this whole paragraph isn't really necessary. The authors could make their point very quickly with one statement that 'The boron isotope-pH proxy is a widely used palaeoceanographic tool (a few example references)."

We appreciate the advice and do not dwell on their work anymore but included them in the references as we should do justice to the pioneers as well.

Line 111-113: Which studies use this approach?

That sentence was wrong and is part of the approach used by Pearson and Palmer (2000). We have changed the text accordingly. “Another approach is based on the assumption that seawater $[Ca^{2+}]$ 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 now be estimated by assuming that increases in A_T with depth were the same as in the modern ocean.

Line 113-114: Surely a changing CCD depth wouldn’t invalidate the approach strictly speaking, it would just mean you can’t use one estimate for a whole long term time series- you have to estimate for each data point?

correct. We have added the fact that Pearson and Palmer (2000) note themselves that the CCD record for the Palaeogene Pacific Ocean is relatively poorly constrained.

Line 95: proven, rather than proved.

done

Line 107: I may be missing something but I’m not sure I see the link with the hydrological cycle?

We have rephrased this to be more precise: “However, salinity and alkalinity may be decoupled in space and time through weathering and changes in riverine alkalinity input.” For example, continental weathering was probably more intense during periods of warm climate and high pCO2, which would deliver more Ca and alkalinity to the ocean.

Lines 93-114: I’m not convinced that the authors are right to present alkalinity as such a paralysingly big problem as the tone of this passage suggests- it surely depends what the goal is. If the goal is to reconstruct CO2 changes, yes alkalinity introduces some uncertainty and it would be better if we knew it, but alkalinity has a relatively small influence on pCO2 values reconstructed from pH, and many studies can just factor in the uncertainty on these estimates into their error propagation. I would suggest the authors reword this somewhat to just make the point that knowing the second parameter would be great to reduce uncertainty in CO2 estimates, rather than present it as such a very acute problem with the proxy. The other question is whether the propagated uncertainty in alkalinity reconstructed from a second proxy (taking into account measurement and calibration uncertainty) would be any lower than the margin of error that can be garnered from things like CCD depth. In reality, given the error bars in figure 2 for example, it would probably be just as large.

We disagree with Michael Henehan that “alkalinity introduces some uncertainty”. The change in surface water $[CO_2]$ is twice as much when the same atmospheric pCO_2 is reached solely via a change in alkalinity as in the coral reef hypothesis (Lea et al., 1999).

We do agree that the propagated uncertainty in the second parameter, reconstructed from an independent proxy (taking into account measurement and calibration uncertainty) might not be any lower than the margin of error that is inherent to assumptions around e.g. total alkalinity

We have added the following text to the manuscript:

"Although $d^{11}B$ has proven to be a reliable proxy for pH and one can argue that ocean pH is the main driver of the past atmospheric CO_2, it is important to remember that changes in past glacial interglacial atmospheric pCO_2 can be achieved via two end-member scenarios (e.g. Sanyal and Bijma, 1999; Lea et al., 1999). In the first scenario, changes in carbonate chemistry are brought about by changes in DIC 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 to pCO2 decrease depends on surface ocean alkalinity (Lea et al., 1999). A smaller change is associated with the drawdown of DIC under conditions of unchanging alkalinity (e.g. strengthening the biological pump without calcite compensation). The change in surface water $[CO_2]$ is twice as much when the same pCO_2 is reached solely via a change in alkalinity as in the coral reef hypothesis (Lea et al., 1999). This is nicely demonstrated in Fig. 1.1.3 of Zeebe and Wolf-Gladrow (2001) and Fig.1 of Foster and Rae (2016).

The real oceans operate somewhere between these endmember 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. Although it has been questioned if changes in the $CaCO_3$:POC rain ratio of biogenic material produced in the surface ocean are directly communicated to the sediments (Ridgwell, 2003), we still believe that knowing 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 e.g. total alkalinity.

Line 130: This is not correct. See for example Fig. 2 of Allen et al. (2012), where pH is kept constant but carbonate ion concentration is varied. Indeed, the authors state that pH was kept constant but carbonate ion increased in Line 128. It's not clear to me what the distinction is between the decoupled chemistry of Allen et al and that of Kaczmarek et al? Allen et al. raised [DIC] and tweaked pH via acid and base addition, so did these authors- what's the big difference?

You are absolutely right. Line 130 as stated is confusing and not correct. What we basically meant to say is that Allen et al (2012) did not vary pH at constant $[CO_3^{2-}]$.

The text has been changed accordingly: "However, they did not decouple pH and $[CO_3^{2-}]$ both ways. In their experiments, they kept pH constant and varied $[CO_3^{2-}]$ but did not vary pH at constant [CO_3^{2-}], leaving the question open whether the B/Ca ratio in planktonic foraminifera is only a function of the ratio between [$B(OH)_4^-$] and C_T or [HCO_3^-] or perhaps also modulated by pH or [CO_3^{2-}].

The manipulations used in our study and Kaczmarek et al. (2015) are exactly the same as Allen et al. (2012). Except that we added experiments at constant [CO_3^{2-}] and varied pH. In addition, we used 10X boron and did not prepare media at constant, ambient, pH and lower [CO_3^{2-}].

Line 136-138: The authors state here that they believe that this is due to a covariation of nutrients and other carbonate system parameters. I would urge them to read the paper again- since the aim of this paper was to test the carbonate system control on B/Ca, we of course tested carbonate system parameters explicitly and B/Ca ratios show no correlations with carbonate system parameters. We also tested for covariation of phosphate with carbonate system parameters (some of these plots are given in the paper) and they show no relationships (p values greater than 0.05, and R2 values <0.1. On this point, the authors are mistaken, and this must be removed. What's more, the findings of the paper, which are directly contrary to the idea that B/Ca can be used in open ocean settings to derive a second carbonate system parameter, should be properly incorporated into the discussion (as with the findings of Babila et al. and Salmon et al.). I am happy to answer any questions the authors have to address any misconceptions about this study.

Point well taken. Our comment related to a possible correlation between PO_4 and B/Ca as suggested by Henehan et al. (2015) was way too short and didn't do justice to your paper were you tested carbonate system parameters explicitly and B/Ca ratios show no correlations with carbonate system parameters.

We were not referring to bulk ocean conditions but rather to their ambient environment. As explained above (under 5), forams, and especially symbiont bearing planktonic forams **never** "see" the bulk ocean carbonate chemistry (see e.g micro-electrode studies by Rink et al. (1998) and the modelling study by Wolf-Gladrow et al. (1999)). They only "see" their ambient carbonate chemistry as modulated by their own life processes as well as by symbiont photosynthesis and respiration (therefore all calibrations are purely empirical and not mechanistic).

We have changed the wording to:

"Recently, Henehan et al. (2015) demonstrated a very clear and close relationship between B/Ca and carbonate chemistry parameters (pH; [B(OH)4-]/[HCO3-] and [B(OH)4-]/DIC) in *Globigerinoides ruber* from culture experiments. However, this relationship is completely lost in the plankton tow samples and the sediments they analyzed. While they explicitly tested for a carbonate chemistry control on B/Ca, they found a strong relationship to [PO4-] and neither a correlations with carbonate system parameters nor a covariation of phosphate with carbonate system parameters. They concluded that apparently B/Ca in *Globigerinoides ruber* is controlled by [PO4-].

We believe that the primary, mechanistic, relationship explaining B/Ca is probably still controlled via carbonate chemistry parameters in the ambient environment of the foraminifer but that it is masked in the field and decoupled from the bulk seawater carbonate chemistry. It should be noted that foraminifera, and especially symbiont bearing planktonic foraminifers never "see" the bulk ocean carbonate chemistry (e.g. e.g micro-electrode study by Rink et al. (1998) and the modelling study by Wolf-Gladrow et al. (1999)). They only "see" their ambient carbonate chemistry as modulated by their own life processes and symbiont photosynthesis and respiration (so called "vital effects"). Existing calibrations and field relationships are therefore purely empirical and not mechanistic.

Here we are specifically focusing on the primary controls of boron uptake and conducted experiments with a the planktonic foraminifer *Orbulina universa* and decoupled pH and [CO_3^{2-}] in the same way as Kaczmarek et al. (2015b). We show that, in principle, combined measurements of $\delta^{11}B_{calcite}$ and B/Ca of the same species as conducted in our study might be used to fully constrain the carbonate chemistry in Earth history. However, based on recent publications (Allen and Hönisch (2012); Babila et al. (2014); Henehan et al. (2015) and Salmon et al. (2016)) it becomes increasingly clear that B/Ca may not be a very robust proxy in the field."

Line 193: How were the seawater d11Bs measured? This is presumably a different way from the foraminiferal shells, and so there is the potential for the different analytical approaches to introduce absolute offsets here. These details are critical if we are to evaluate these numbers in an absolute sense, and need to be added. Measurements of natural seawater could also be added to give a feel for the typical reproducibility of the approaches for a standard everyone has.

We have added a method section on SW boron analysis and added the individual SW analyses to the supplement

Line 197: It's not clear to me why Martinez-Boti should be cited here for a salinity of 38.

Not clear to us either, must have been some glitch. Has been taken out

Line 261: Faraday should be capitalized

Done

Lines 306, 310: 'seawater scale' is a particular definition of pH, and not to do with boron. Better to say 'normalised to natural seawater'.

Very good point. Has been corrected.

Line 342 (and Fig. 3): The choice of uncertainty calculation here seems wrong. The authors show that the repeat measurements of foram d11B within a test vary by far more than the calculated internal error of a single measurement described in Equation 2. It is therefore not reasonable to apply the single measurement uncertainty estimate to the 'whole test' d11B value, as it only accounts for the measurement uncertainty and not the variability between measurements. The best approximation of the uncertainty of the 'true' average bulk-test boron isotope ratio would in this case be 2 standard deviations of the variability within the test- these should be the error bars on Fig. 3 if they are to be truly representative of true variability. On top of this, when presenting the data in ‘normal d11Bsw’ space, the authors should also propagate the uncertainty stemming from the uncertainty in experimental seawater d11B- since these values are also critical and the uncertainty on these numbers is very large in some cases.

Each data point respresents one single raster ablation of one single specimes (i.e. “whole tests”). The major difference between LA and “wet chemistry” data is that the latter method averages individual variability before analysis by analyzing multiple, dissolved, shells in one go, while LA captures individual variability (which is large and real as argued above) and averages afterwards. Regarding the error propagation of the “uncertainty” stemming from individual LA measurements and the analytical uncertainty from the seawater $d^{11}B$ analysis we have added table 4 in the supplement. The propagated error is of course large as it includes the individual $d^{11}B$ variability. We like to point out that this variability is data/information which is not related to the analytical uncertainty. We have added a calcite vs borate $d^{11}B$ crossplot (Fig. 4) to avoid the conversion into the seawater scale and making the error propagation obsolete. However, since not all studies (cited in Fig. 3) report the parameters required for the calculation of $d^{11}B$ of borate, we kept figure 3 but did not plot the propagated error.

Line 355: Foster identify a relationship between carbonate ion and KD, not strictly B/Ca ratios. This is a really important distinction since Allen and Hönisch (2012) point out that the way KD is calculated can drive a correlation without any coherent pattern in raw B/Ca ratios.

Good point. Has been changed accordingly.

New text: “Foster (2008) showed that the partition coefficient for the B/Ca ratio is influenced by $[CO_2^{-3}]$ (and temperature). Although complicating the application as a proxy related to $[B(OH)_4^-]/[HCO_3^-]$, he also demonstrated that B/Ca in combination with $d^{11}B$ can be used to fully constrain the carbonate system in downcore records. Nonetheless, he identified $[CO_3^{2-}]$ as having a major (secondary) control on B/Ca in samples of foraminifera from down core samples and core tops.”

Line 360-365: This is what Allen et al. (2012) did, and the conclusions reached are largely the same as this study. It seems therefore odd to mention two older studies in this paragraph first in Foster (2008) and Allen et al. (2011), but not mention the more relevant study right from the off. It has the effect of almost suggesting this study is the first to do this, but in fact it is largely replicating what Allen et al. (2012) did.

This is not quite true. As argued before, even though the manipulations used in our study and Kaczmarek et al. (2015) are largely the same as Allen et al. (2012), we have added experiments at constant $[CO_3^{2-}]$ and varied pH, to decouple both the other way around. In addition, we also analysed $d^{11}B$, next to B/Ca.

Line 376-379: What is Fig. 5.1C? The discussion is a little odd here. Since the argument with competition is that B/Ca will correlate with the ratio of borate to carbon species, then these observations are to be expected: changing carbonate ion without changing pH changes the denominator, and changing pH without carbonate ion changes the numerator in altering boron speciation. So these are two sides of the same coin.

Thanks for pointing this out. Fig. 5.1C should be Fig. 2E.

You are right that the two opposing experiments could be seen as two sides of the B/Ca coin, where one manipulation affects the denominator and the other the numerator but in a chemical sense these experiments are very different. Biologically, the impact is very different as well. *A priory* we expect very different responses as the two opposing experiments will impact the ambient carbonate chemistry differently and therefore change the extent of the "vital effects". The ion channels taking up calcium and carbon during calcification may behave differently. [CO_3^{2-}] changes at constant pH probably affect calcification rate, while constant [CO_3^{2-}] at variable pH will not affect calcification rate, etc.

Line 391-392: This sentence as it is currently phrased suggests that Yu et al. (2007) would support bicarbonate being critical here rather than DIC or carbonate ion. However, this is not strictly true: Yu et al. (2007) never tried to regress against borate/DIC in that paper.. Just because they present borate/bicarbonate in this paper, it doesn't rule out the possibility that the correlation with borate/DIC or borate/carbonate might have been stronger. This passage therefore needs to be rewritten.

We have rewritten this passage:

"Although analysis of planktonic foraminifera from core tops revealed a good correlation between B/Ca and [$B(OH)_4^-$]/[HCO_3^-] it doesn't rule out a possible correlation with $B(OH)_4^-$/$CO_3^{2-}$and/or $B(OH)_4^-/C_T$.) (Yu et al., 2007). "

Line 412-414: Is there any a priori reason for us to ever expect this? If so, it might be good to give it here.

A priori no, but we did vary [CO_3^{2-}] at constant pH and found, surprisingly, an effect of [CO_3^{2-}] on d^{11}B (see answer to reviewer 1)! This was previously not discussed in the manuscript but we have added text:

"d^{11}B increases with increasing [CO_3^{2-}] at constant pH_T from 17.2‰ at 238 µmol/kg CO_3^{2-} to 19.9‰ at 534 µmol/kg 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 difference between the mean d^{11}B values of the [CO_3^{2-}] treatments 239 and 286 µmol/kg were close to significance but only between 239 and 534 µmol/kg the difference was significant (Supplement Table 3). Because, this range in [CO_3^{2-}] is beyond that of the real ocean and because pH and [CO_3^{2-}] co-vary, we believe that this observation is only important for a better understanding of the d^{11}B controls and does not significantly impact existing calibrations."

Line 423: Need to be clearer here – values for what? Borate ion.

We have changed the sentence: “The δ^{11}B values for *O. universa* found in this study match closely with the δ^{11}B values of borate ion in artificial seawater given by Klochko et al. (2006).”.

Lines 437-441: Note again, this is fine in culture, but is ignoring plenty of open ocean data that suggests that the control of HCO3- on B/Ca is overwhelmed by competing controls.

We have added a large paragraph in the section “proxy implications”:

“A wide range of [HCO_3^-] was necessary to facilitate de-coupling the carbonate system from pH_T. The high [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 correlated to [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 e.g. [$B(OH)_4^-$]/[HCO_3^-]. Based on plankton tow, sediment trap and core-top data, they concluded that, apparently, B/Ca in *G. ruber* is controlled by [PO_4^{3-}]. However, it should be noted that foraminifera, and especially symbiont bearing planktonic foraminifers never “see” 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 “see” their ambient carbonate chemistry as modulated by their own life processes and 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 symbiont bearing planktonic Foraminifera, *G. ruber* is probably the most "autotropic" (Bijma et al., 1992). Although we cannot prove it, we assume that symbiont photosynthetic rates are higher at elevated [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 [CO_3^{2-}], [HCO_3^-]) may still hold up. At this point, there is no obvious direct link between [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., (2015b) 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, 1965; Bé et al., 1982; Caron et al., 1981; Spero and Parker, 1985; Jørgensen et al., 1985; Hemleben et al., 1987) has clearly demonstrated the huge impact of symbionts on foraminiferal shell growth. Interestingly, Babila et al. (2014) write: "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 species (*G. bulloides* and *G. inflata*) and even the symbiont bearing species *G. sacculifer* are related to [CO_3^{2-}] and [$B(OH)_4^-/HCO_3^-$]. 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 [CO_3^{2-}] variability (or borate/bicarbonate) is small (ca. 20 µmol kg^{-1} in the depth range 30 to 50m) 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 also very close to d^{11}B of borate, suggesting that their "vital effects" are muted in the real ocean, especially the symbiont impact of raising the calibration curve above d^{11}B of borate. This is supported by the observation of Hemleben et al., (1994) that *O .universa* occupies a subsurface maximum (in the Red Sea) between 20-60 meters (Hemleben et al., 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 field studies are not suitable for elucidating the mechanisms of proxy incorporation but are very helpful in showing which species best suited for paleo reconstructions of carbonate system parameters and if and how much vital effects determine species specific offsets from the target parameters. We agree with Henehan et al. (2015) that *G. ruber* is not a good choice as its primary relationship to carbonate chemistry parameters is not very robust. However, other symbiont bearing species, non-symbiotic planktonic Foraminifera and deep sea benthics, may still be a viable option to use B/Ca for carbonate chemistry reconstructions."

Lines 462-464: Again, this is suggesting that these culture observations can be transferred to the open ocean when a number of more recent studies that the authors do not cite here (and should) show that these relationships don't hold up outside of the lab.

see above

Fig 3: In panel A, these data from other studies are all plotted on one d11B-pH plot. But critically, pK*B differs between each study. Therefore some of the variation in behavior of d11B with pH in each study can derive from a different pK*B in each case. This is why we have moved towards plotting things in d11Bcalcite-vs-d11Bborate space. To represent these data in an informative way, each datapoint needs to be normalised to a single pK*B, which is clumsy to try and do. I would advise that the authors plot these data in a calcite vs borate d11B crossplot instead. How also were the lines constructed between points? Also, as mentioned above, error bars should also account for the uncertainty in d11Bsw that is carried through into these normalised d11B values. Why are there no error bars on pH, also? There should be. Finally, I see no benefit in plotting the Kakihana et al borate ion curve in panel A at all- this value is defunct, and has been shown to be erroneous (Rustad et al. 2010), so why plot it?

We have added a calcite vs borate d^{11}B crossplot (Fig. 4) and added some text in the method section:

“One could further argue that the uncertainty stemming from the analysis of culture water d^{11}B should also be propagated when plotting in ‘normal d^{11}B$_{sw}$’ space (supplementary table 4). The propagated error is of course large as it includes the individual d^{11}B variability of the foraminifers. We like to point out that this variability represents true data which is largely unrelated to analytical uncertainty. We have added a calcite vs borate d^{11}B crossplot (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 d^{11}B of borate we plotted for comparison in ‘normal d^{11}B$_{sw}$’ space but did not propagate the error related to the analysis of culture water d^{11}B.”.

We have added the error bars on pH

We leave the d^{11}B borate curve with a = 20 permil (but do not mention Kakihana) in Fig. 3 for comparison of slope (which is less steep than the Klochko curve)

Howes EL [1,2], Kaczmarek K [1], Raitzsch M [3], Mewes A [1], Bijma N [4], Horn I [5], Misra S [6], Gattuso J-P [2,7], Bijma J [1]

[1.] Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research, Bremerhaven, Germany
[2.] Sorbonne Universités, UPMC Univ Paris 06, Observatoire Océanologique, F-06230 Villefranche-sur-mer, France
[3.] MARUM – Zentrum für Marine Umweltwissenschaften, Universität Bremen, Leobener Str., 28359 Bremen, Germany

[4.] Christian-Albrechts-Universität zu Kiel, Germany
[5.] Institut für Mineralogie, Leibniz Universität Hannover, Callinstraße 3, 30167 Hannover, Germany
[6.] University of Cambridge, Department of Earth Sciences, Godwin Laboratory for Paleoclimate Research, Downing Street, Cambridge, United Kingdom
[7.] CNRS-INSU, Laboratoire d'Océanographie de Villefranche, F-06230 Villefranche-sur-mer, France

Ella Howes 11/11/2016 17:22
Formatted: Width: 20.99 cm, Height: 29.7 cm, Numbering: Restart each page

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 ($\delta^{11}B$) of planktonic foraminifera shells is a powerful tool to reconstruct changes in past surface ocean pH. As $B(OH)_4^-$ is substituted into the biogenic calcite lattice in place of CO_3^{2-} and both borate and carbonate ion are more abundant at higher pH, it was suggested early on that B/Ca ratios in biogenic calcite may serve as a proxy for $[CO_3^{2-}]$. 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 $[CO_3^{2-}]$ 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 $[CO_3^{2-}]$ and to investigate their impact on the B/Ca ratio and $\delta^{11}B$, we conducted culture experiments with the planktonic foraminifer *Orbulina universa* in manipulated culture media: constant pH (8.05), but changing $[CO_3^{2-}]$ (238, 286 and 534 µmol kg^{-1} CO_3^{2-}) and at constant $[CO_3^{2-}]$ (276 ±

19.5 µmol 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 an MC ICP-MS. Our results show that, as expected, δ^{11}B is mainly controlled by pH but it is also modulated by [CO_3^{2-}]. On the other hand, the B/Ca ratio is driven by [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 [HCO_3^-] can be obscured by other environmental parameters.

Ella Howes 9/11/2016 21:37
Deleted: the

Introduction

Before the Anthropocene, the atmospheric CO_2 concentration was governed by the surface ocean [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 evolution of atmospheric pCO_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).

Ella Howes 9/11/2016 21:24
Deleted: other

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

Ella Howes 9/11/2016 21:25
Deleted:

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 $\delta^{11}B$ of biogenic carbonates alone with assumptions regarding a secondary parameter. In these reconstructions, total alkalinity (A_T) or [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; Dickson, 1992; Wolf-Gladrow et al., 1999; Wolf-Gladrow et al., 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 [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".

Ella Howes 9/11/2016 21:26
Deleted: In the modern ocean,

Ella Howes 9/11/2016 21:29
Deleted: , because total alkalinity is the charge balance between the major conservative ions in seawater, and this charge balance varies with salinity

Ella Howes 9/11/2016 21:29
Deleted: total alkalinity is a conservative parameter. It

Ella Howes 9/11/2016 21:29
Deleted: so that

Ella Howes 9/11/2016 21:31
Deleted: , themselves,

Although $\delta^{11}B$ 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 pCO_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

Ella Howes 9/11/2016 21:41
Deleted: the

Ella Howes 10/11/2016 18:27
Deleted: changes in DIC

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 pCO_2 decrease, depends 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 $[CO_2]$ is twice as much when the same atmospheric pCO_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).

Ella Howes 9/11/2016 21:45
Deleted: DIC

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 (2015) 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 ($B(OH)_3$) and borate ion ($B(OH)_4^-$; 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 *p*H (>10), boron primarily exists as borate. Because of the isotopic fractionation between the two aqueous species (Fig 1B; $\alpha_{4-3} = R_{B(OH)4-}/R_{B(OH)3}$), the boron isotopic composition of each species is also pH dependent (Hemming and Hanson, 1992; Palmer et al., 1987; Sanyal et al., 1996; Sanyal et al., 2000). $B(OH)_3$ is enriched in the stable isotope ^{11}B compared to $B(OH)_4^-$, with a constant isotopic fractionation of 27.2 ‰ between the two boron species (Klochko et al., 2009;

Ella Howes 9/11/2016 21:46
Deleted: F

Ella Howes 9/11/2016 21:47
Deleted:

Klochko et al., 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 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 $\delta^{11}B$ based pCO_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 [CO_3^{2-}] (Foster, 2008); however, given that the concentration of borate $B(OH)_4^-$) increases with pH and pH co-varies with [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 disentangle 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 [HCO_3^-]) at constant pH (i.e. [$B(OH)_4^-$] was constant while [CO_3^{2-}] and [HCO_3^-] were increased), suggesting that borate and carbon species compete for the inclusion into the calcite lattice. In their experiments, they kept pH constant and varied [CO_3^{2-}]

Ella Howes 9/11/2016 21:49
Deleted:)

but did not vary pH at constant [CO_3^{2-}], leaving the question open whether the B/Ca ratio in planktonic foraminifera is only a function of the ratio between [$B(OH)_4^-$] and C_T or [HCO_3^-] or perhaps also modulated by pH or [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 [$B(OH)_4^-$] and [HCO_3^-], rather than by pH or [CO_3^{2-}].

Recently, Henehan et al. (2015) demonstrated a very clear and close relationship between B/Ca and carbonate chemistry parameters (pH; [$B(OH)_4^-$]/[HCO_3^-] and [$B(OH)_4^-$]/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 [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 [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.

Ella Howes 9/11/2016 21:52
Formatted: Font:(Default) Times New Roman

Ella Howes 9/11/2016 21:52
Deleted: DIC

Here, we are specifically focusing on the primary controls of boron uptake and conducted experiments with a the planktonic foraminifer *Orbulina universa* and decoupled pH and [CO_3^{2-}] in the same way as Kaczmarek et al. (2015b). We show, in principle, that combined measurements of $\delta^{11}B_{calcite}$ and B/Ca on single shells of planktonic foraminifera might be used to fully constrain the carbonate chemistry in downcore 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.

Ella Howes 9/11/2016 21:53
Deleted: F

Ella Howes 9/11/2016 21:54
Deleted: is

Methods

Collection and culturing

Ella Howes 10/11/2016 18:31
Deleted: *g:*

Ella Howes 10/11/2016 18:29
Deleted: *rbulina*

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'Oceanographie 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, measured with a light microscope, and transferred to 0.2 µm-filtered seawater, whose carbonate chemistry was accurately determined and subsequently modified. Specimens were maintained individually in air-tight 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}.

Ella Howes 9/11/2016 21:55
Deleted:

The foraminifers 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 deionised 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 hours, rinsed four times using de-ionized 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.

Ella Howes 11/11/2016 17:22
Comment [1]: Added in resonse to Rev 2 comment: Have the foraminifera shells been treated chemically prior to laser analyses (e.g.oxydative cleaning)?

Modified seawater chemistry

The objective of these experiments was to decouple seawater pH and [CO_3^{2-}] 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 [CO_3^{2-}], seawater carbonate chemistry was modified manipulating pH_T, using NaOH and HCl, and dissolved inorganic carbon (C_T) by adding gravimetrically carbonate and bicarbonate or bubbling with CO_2. 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; Sanyal et al., 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, respectively. 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. pH of the culture solutions was measured using a Metrohm, 826 mobile pH meter with 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 $HgCl_2$ pending determination of dissolved inorganic carbon (C_T). Samples were measured using an AIRICA (Marianda, Kiel) fitted with a Licor 6262 infra-red 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).

Ella Howes 9/11/2016 21:57
Deleted:

Ella Howes 10/11/2016 18:31
Deleted:

Ella Howes 9/11/2016 21:58
Deleted: was

Ella Howes 10/11/2016 18:31
Deleted:

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 ($^{11}B/^{10}B = 4.04362 \pm 0.00137$) (Catanzaro et al., 1970) where:

$$\delta^{11}B_{sample}(‰) = \left[\frac{\left(^{11/10}B\right)_{sample}}{\left(^{11/10}B\right)_{NISTSRM951a}} - 1\right] \times 1000$$

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 ^{11}B measurements of seawater (from the Atlantic Ocean) and secondary boron standards (AE 120, 121, 122) with published (accepted) results. Our estimates of $\delta^{11}B_{SW}$ of 39.8 ± 0.4‰ (2s, n = 30) are independent of sample size and are in agreement with published values of 39.6 ± 0.04‰ (Foster et al., 2010) and 39.7 ± 0.6‰ (Spivack and Edmond, 1987). Moreover, our $\delta^{11}B$ estimates of SRM AE-120 (-20.2 ± 0.5 ‰, 2s, n = 33), SRM AE-121 (19.8 ± 0.4 ‰, 2s, n =16), SRM AE-122 (39.6 ± 0.5 ‰, 2s, 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).

Ella Howes 11/11/2016 17:22

Comment [2]: Added in reponse to Rev 2 comment:
I assume d11B of the culturing water has been measured, as the data are provided in table 1. I do miss information on how these data have been generated. I would assume using MC-ICPMS, relative to NBS SRM-951?

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 build 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 $\delta^{11}B$ of *O. universa* can be found in Kaczmarek et al. (2015a). A summary of the procedure is given below.

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

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%, $CaCO_3$: 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).

Ella Howes 9/11/2016 22:03

Deleted: The correction for different laser repetition rates was realized by the analysis of calcium in the reference material and in the sample because their Ca concentrations are known (NIST SRM 610: 8.45%, $CaCO_3$: 40%) using the optical spectrometer. More information on this procedure is provided

Calcium analysis

The Maya2000 Pro is a high-sensitivity fiber optical spectrometer. It has a measuring range between 250 to 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 2D FFT-CCD detector, and a grating with a groove density of 1200 lines/mm. The optical fiber used is two meters 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 spectra 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.

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 (Molectron, USA) are 3.2 mJ/pulse at

777 nm, 0.7 mJ/pulse at 259 nm, and 0.085 mJ/pulse at 194 nm. After the fourth harmonic generation stage, the 194 nm beam is steered by eight dichronic mirrors into an 8x 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 ~2 J/cm^2 is maintained. Reference material measurements were performed in raster mode (100 µm x 100 µm) at 10 Hz, 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) (Hergenröder and Hommes, 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 δ^{11}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 δ^{11}B *O. universa* and δ^{11}B of $B(OH)_4^-$, the isotopic difference between the two standards can be neglected and it does not make a difference if values are reported versus one or the other standard.

Boron Isotope Analysis - Acquisition parameters

Ella Howes 11/11/2016 17:22
Comment [3]: Added in response to Henehan’s comment:

Ella Howes 11/11/2016 17:22
Comment [4]: In response to Rev 1 comment: “d11B data should be normalized to NIST SRM 951, not NIST SRM 610, as most of the cited reports of the literature do.”

Ella Howes 11/11/2016 17:22
Comment [5]: Added in respose to Rev 2 comment:
The water composition is massively modified (10xB concentration and about 35 permill lighter than natural seawater). That’s fine, the normalization should allow for comparability of the data. So, water data are presumably expressed relative to NBS951 (conventional delta11B notation). LA-MC-ICPMS data relate to NIST SRM-610. Nothing is mentioned about any further normalization, regarding differences between both SRMs. It appears the authors assume both to have identical boron isotopic composition. When using a standard of an entirely different matrix during the laser analysis of foraminifera (silicate vs. carbonate), and ablating quite different amounts of both, some justification is needed to convince readers that no offsets (analytical artefacts) compromise the data.

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 (CDD, 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 $^{40}\mathrm{Ar}^{4+}$ or $^{20}\mathrm{Ne}^{2+}$, 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 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/sample took four minutes 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:

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

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:

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

Where $^{11/10}$B ratios represent mean values of the reference material and the sample calculated from one measurement, respectively (based on 160 cycles) and SE represents the standard error of the $^{11/10}$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:

$$\delta^{11}B_{NIST610}(‰) = \left[\frac{^{11/10}B_0}{\left(^{11/10}B_{-1}+^{11/10}B_{+1}\right)/2} - 1\right]\times 1000 \qquad \text{Eq. (3)}$$

Where the measurements of the $(^{11/10}B)_{-1}$ and $(^{11/10}B)_{+1}$ ratios of NIST 610 were performed before and after the measurement of $(^{11/10}B)_0$, 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‰.

Conversion of $\delta^{11}B_{O.\ universa}$ to natural seawater

Due to the additional B addition to our culture media the $\delta^{11}B_{seawater}$ shifted from 37.963 (Mediterranean), to, on average, 4.66 ‰ (Table 1). Therefore, the $\delta^{11}B_{O.\ universa}$ shifted accordingly. In order to compare our *O. universa* data to published values (Fig. 3A), the measured δ^{11}B from each experiment was normalized to natural seawater using (Zeebe and Wolf-Gladrow, 2001):

Ella Howes 9/11/2016 22:10
Deleted: 5

$$\delta^{11}B_c = \alpha_{sw-msw}\times\delta^{11}B_m + \varepsilon \qquad \text{Eq. (4)}$$

Where ε is (α_{sw-msw} -1) x 1000, $\delta^{11}B_c$ represents the converted δ^{11}B for the measured value ($\delta^{11}B_m$), α_{sw-msw} is the fractionation factor expressing the difference between the natural seawater and manipulated seawater:

$$\alpha_{sw-msw} = (\delta^{11}B_{sw} + 10^3)/(\delta^{11}B_{msw} + 10^3) \qquad \text{Eq. (5)}$$

Statistics

Ella Howes 10/11/2016 18:33
Deleted: s:

Lamtool (a modified Excel spreadsheet, initially programmed by Jan Kosler, University of Bergen, Norway) was used for analysis and background correction of the $\delta^{11}B$ data. All other statistics were carried out using R (R Core Team, 2008). Error bars represent ± 2σ 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 $\delta^{11}B$ 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 $\delta^{11}B$ variability in *Cibicidoides wuellerstorfi* is up to ca. 10‰ (Sadekov et al., 2016), while the inter-specimen $\delta^{11}B$ variability of *Amphistegina lessonii* from the same treatment is ca. 6‰ (Kaczmarek et al., 2015b). Histograms of single-foram $\delta^{11}B$ measurements from each of our pH treatments (supplementary Fig. 1) 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 (supplementary Fig. 1) 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.

Ella Howes 9/11/2016 22:12
Deleted: As impressively demonstrated by

Ella Howes 9/11/2016 22:12
Deleted: is

Ella Howes 9/11/2016 22:13
Deleted: ring

Ella Howes 9/11/2016 22:14
Deleted: , t

Ella Howes 11/11/2016 17:22
Comment [6]: Added in response to Henehan’s comment:

One could further argue that the uncertainty stemming from the analysis of culture water $\delta^{11}B$ should also be propagated when plotting in ‘normal $\delta^{11}B_{sw}$’ space (supplementary table 4). The propagated error is, of course, large as it includes the individual $\delta^{11}B$ 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 $\delta^{11}B$ crossplot (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 $\delta^{11}B$ of borate, we plotted for comparison in 'normal $\delta^{11}B_{sw}$' space (Fig. 3A) but did not propagate the error related to the analysis of culture water $\delta^{11}B$.

Results

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 [CO_2] but to a lesser extent ($R^2 = 0.64$; Fig 2C). B/Ca also decreases with increasing [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 [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 [HCO_3^-], irrespective of the pH_T of the culture medium ($R^2 = 0.96$; Fig 2G). Plotted against the ratio of [$B(OH)_4^-$] over each of the carbon species (Fig 2B, D, F, H), the correlations are high for all combinations but highest for [$B(OH)_4^-$]/[CO_3^{-2}]. Based on first principles, we predict a positive correlation between B/Ca and $\delta^{11}B$ because at higher pH, not only does the isotopic composition of borate become heavier, but its concentration also increases. Figure 2A (supplement) shows the individual B/Ca, $\delta^{11}B$ 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 $\delta^{11}B$ 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 $\delta^{11}B$ in some of the treatments, we believe that the individual B/Ca, $\delta^{11}B$ pairs within a treatment are uncorrelated. However, the average values for the four treatments with [CO_3^{-2}] between 238 – 297 µmol kg^{-1} do show a positive correlation between B/Ca and $\delta^{11}B$. The "outlier" (treatment at pH 8.05;

Ella Howes 9/11/2016 22:16
Deleted:

Ella Howes 10/11/2016 18:35
Deleted: :

Ella Howes 10/11/2016 18:34
Deleted: *:*

Ella Howes 9/11/2016 22:17
Deleted:

Ella Howes 9/11/2016 22:18
Deleted: s

Ella Howes 9/11/2016 22:18
Deleted:

Ella Howes 9/11/2016 22:19
Deleted: demonstrate

Ella Howes 9/11/2016 22:19
Deleted: , h

[CO_3^{-2}] = 534) can be explained by the high [HCO_3^-], relative to the other pH = 8.05 treatments.

Ella Howes 11/11/2016 17:22
Comment [7]: In response to Rev 1 comment: Is there any correlation between B/Ca and d11B?

Ella Howes 10/11/2016 18:35
Deleted: :

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

Single, measured δ11B values of *O. universa* are given in Supplementary Table 1, errors are calculated according to Eq. (2). Median and converted values using Eq. (4) and Eq. (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 pH_T 7.7 to 18.8‰ at pH_T 8.05. These values are close to the B fractionation curve of $B(OH)_4^-$ obtained for artificial seawater by Klochko et al. (2006; Fig 3A). $\delta^{11}B$ increases with increasing [CO_3^{2-}] at constant pH_T from 17.2‰ at 238 μmol/kg CO_3^{-2} to 19.9‰ at 534 μmol/kg 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 $\delta^{11}B$ values of the [CO_3^{2-}] treatments 239 and 286 μmol/kg were close to significance but only between 239 and 534 μmol/kg; the difference was significant (supplementary table 3). Because this range in [CO_3^{2-}] is beyond that of the real ocean and because pH and [CO_3^{2-}] co-vary, we believe that this observation is only important for a better understanding of the $\delta^{11}B$ controls and does not significantly impact existing calibrations.

Ella Howes 9/11/2016 22:20
Deleted: is

Ella Howes 11/11/2016 17:22
Comment [8]: In response to Rev 1 comment: “In spite of the large analytical error, it is difficult to conclude that no significant effect of [CO3 2-] on d11B was found.”

Ella Howes 10/11/2016 18:35
Deleted: :

Ella Howes 10/11/2016 18:35
Deleted: :

Discussion

B/Ca

Foster (2008) showed that the partition coefficient for the B/Ca ratio is influenced by [CO_2^{-3}] (and temperature). Although complicating the application as a proxy related to [$B(OH)_4^-$]/[HCO_3^-], he also demonstrated that B/Ca in combination with $\delta^{11}B$ can be used to fully constrain the carbonate system in downcore records. Nonetheless, he identified [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 [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 ($[B(OH)_4^-]/[HCO_3^-]$), 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.

Ella Howes 9/11/2016 22:23
Deleted: B

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 into the calcite lattice. When B/Ca ratios are plotted against $[CO_2]$, the relationship is similar to that of C_T, however, only <1% of C_T is in the form of CO_2 so this species is unlikely to have a major control on boron incorporation. The remaining >99% is ~10% CO_3^{2-} and ~ 90% HCO_3^- (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 HCO_3^- and CO_3^{2-} as substrate for calcification and, therefore, that C_T is the factor controlling the B/Ca ratios. However, because $[HCO_3^-]$ and $[CO_3^{2-}]$ 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 $[CO_3^{2-}]$ (Fig. 2E) supports the hypothesis of competition between CO_3^{2-} and $B(OH)_4^-$. However, when $[CO_3^{2-}]$ is held constant and pH_T is decreased, B/Ca significantly decreases despite the fact that $[CO_3^{2-}]$ remains more or less constant (Fig 2 E, Table 1). If the same relationships are examined for B/Ca and $[HCO_3^-]$ a strong correlation between $[HCO_3^-]$ and B/Ca is observed for both, the absolute concentration of HCO_3^- (Fig. 2G) and also for the ratio of $[B(OH)_4^-]/[HCO_3^-]$ with no effect of changing pH_T (Fig. 2H). The close correlation between $[CO_3^{2-}]$ and B/Ca at constant pH_T can be explained by the corresponding increases in $[HCO_3^-]$ in these treatments (Table 1).

Ella Howes 9/11/2016 22:25
Deleted:

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 into 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 $[B(OH)_4^-]/[HCO_3^-]$ it does not rule out a possible correlation with $B(OH)_4^-/CO_3^{2-}$ and/or $B(OH)_4^-/C_T$ (Yu et al., 2007).

Ella Howes 9/11/2016 22:25
Deleted: n't

A recent study by Kaczmarek et al. (2015b) shows the same competition between $B(OH)_4^-$ and HCO_3^- in the benthic species *A. lessonii* cultured in a pH-$[CO_3^{2-}]$ decoupled seawater. The observation that B/Ca is driven by $B(OH)_4^-/HCO_3^-$ and not related to CO_3^{2-} 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 $B(OH)_4^-/CO_3^{2-}$.

Ella Howes 10/11/2016 17:41
Deleted:

Ella Howes 10/11/2016 18:38
Deleted: *mphistegina*

The finding that $B(OH)_4^-/HCO_3^-$ 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.

Ella Howes 10/11/2016 17:42
Deleted: is controlling on

$$K_D = \frac{[B/Ca]_{solid}}{[B(OH)_4{}^- / HCO_3^-]_{seawater}} \qquad \text{Eq. (6)}$$

To summarize, based on our study, we can eliminate a control by $[CO_3^{2-}]$ but cannot exclude $[B(OH)_4^-/CO_3^{2-}]$. 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 $[B(OH)_4^-/HCO_3^-]$.

Ella Howes 10/11/2016 18:38
Deleted: *mphistegina*

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

Ella Howes 10/11/2016 18:38
Deleted: :

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 $\delta^{11}B$. 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 $\delta^{11}B$ values increased with increasing $[CO_3^{2-}]$. The $\delta^{11}B$ values for *O. universa* found in this study match closely with the $\delta^{11}B$ 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 10x 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 $[CO_3^{2-}]$ on $\delta^{11}B$ 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 $[HCO_3^-]$ (Fig. 3C).

Ella Howes 10/11/2016 18:00
Deleted: Sanyal et al. (1996)

Ella Howes 10/11/2016 18:01
Deleted: Klochko et al. (2006)

Ella Howes 10/11/2016 18:03
Deleted: X

Ella Howes 10/11/2016 18:04
Deleted:

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 paleoceanographic 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 $\delta^{11}B$ as an independent pH proxy and supports the growing body of evidence that B/Ca in planktonic foraminiferal calcite is mechanistically controlled by $[HCO_3^{2-}]$ (Yu et al., 2007), thereby allowing researchers to fully constrain the carbonate system in combination with $\delta^{11}B$.

Based on our results and other culture studies, it becomes clear that despite strong biological effects on the ambient carbonate chemistry (Köhler-Rink and Kuhl, 2001; Köhler-Rink and Kühl, 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 [HCO_3^-] is the primary control of the B/Ca ratio. The correlation of the B/Ca ratio to [HCO_3^-] rather than to [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 [HCO_3^-] was necessary to facilitate de-coupling the carbonate system from pH_T. The high [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 correlated to [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 e.g. [$B(OH)_4^-$]/[HCO_3^-]. Based on plankton tow, sediment trap and core-top data, they concluded that, apparently, B/Ca in *G. ruber* is controlled by [PO_4^{3-}]. However, it should be noted that foraminifera, and especially symbiont 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 symbiont bearing planktonic foraminifera, *G. ruber* is probably the most "autotropic" (Bijma et al., 1992). Although we cannot prove it, we assume that symbiont photosynthetic rates are higher at elevated [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 [CO_3^{2-}], [HCO_3^-]) may still hold up. At this point, there is no obvious direct link

Ella Howes 10/11/2016 18:08
Deleted: and

Ella Howes 10/11/2016 18:09
Deleted: F

between [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.

Ella Howes 11/11/2016 17:22
Comment [9]: Added in response to Henehan's commnet

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 [CO_3^{2-}] and [$B(OH)_4^-/HCO_3^-$]. 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 [CO_3^{2-}] variability (or borate/bicarbonate) is small (ca. 20 µmol 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. (in press) propose a field calibration for *O. universa* that is very close to $\delta^{11}B$ of borate, suggesting that their "vital effects" are muted in the real ocean, especially the symbiont impact of raising the calibration curve above $\delta^{11}B$ 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 20-60 m (Hemleben et al., 1994; Fig. 5) and could explain why B/Ca in this species is not (completely) masked by symbiont photosynthesis (Salmon et al., 2016).

Ella Howes 11/11/2016 17:22
Comment [10]: Added in response to Henehan's comment:
4) "…….inclusion of measurements of open ocean O. universa…….would test the hypothesis put forward for the apparently muted vital effects." …… " It would also address the issue of bicarbonate control- since there are a number of studies that show that just because these patterns can be seen in culture, it doesn't mean they will hold up outside of the lab."

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 ~380 µmol photons m^{-2} s^{-1}, providing a photon flux for maximum photosynthetic rates (P_{max}) of the symbionts (Spero and Parker, 1985; Spero and DeNiro, 1987; 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 µmol photons m^{-2} 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 et al., 2003) and our laboratory calibration closer to the field calibration of Henehan et al., in press.

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 benthics, may still be a viable option to use B/Ca for carbonate chemistry reconstructions.

Ella Howes 10/11/2016 18:21
Deleted: Laboratory experiments are usually carried out with "pet" Foraminifera but state nothing about their suitability for paleo studies.

Ella Howes 10/11/2016 18:22
Deleted: F

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 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).

References

Allen, K. A. and Hönisch, B.: The planktic foraminiferal B/Ca proxy for seawater carbonate chemistry: A critical evaluation, Earth Planet Sci Lett, 345, 203-211, 2012.

Allen, K. A., Hönisch, B., Eggins, S. M., and Rosenthal, Y.: Environmental controls on B/Ca in calcite tests of the tropical planktic foraminifer species *Globigerinoides ruber* and *Globigerinoides sacculifer*, Earth Planet Sci Lett, 351, 270-280, 2012.

Allen, K. A., Hönisch, B., Eggins, S. M., Yu, J., Spero, H. J., and Elderfield, H.: Controls on boron incorporation in cultured tests of the planktic foraminifer *Orbulina universa*, Earth Planet Sci Lett, 309, 291-301, 2011.

Babila, T. L., Rosenthal, Y., and Conte, M. H.: Evaluation of the biogeochemical controls on B/Ca of *Globigerinoides ruber* white from the Oceanic Flux Program, Bermuda, Earth Planet Sci Lett, 404, 67-76, 2014.

Balan, E., Pietrucci, F., Gervais, C., Blanchard, M., Schott, J., and Gaillardet, J.: First-principles study of boron speciation in calcite and aragonite, Geochim. Cosmochim. Acta, 193, 119-131, 2016.

Bé, A., Spero, H., and Anderson, O.: Effects of symbiont elimination and reinfection on the life processes of the planktonic foraminifer *Globigerinoides sacculifer*, Mar. Biol., 70, 73-86, 1982.

Bé, A. W.: The influence of depth on shell growth in *Globigerinoides sacculifer* (Brady), Micropaleontology, 1965. 81-97, 1965.

Bemis, B. E., Spero, H. J., Bijma, J., and Lea, D. W.: Reevaluation of the oxygen isotopic compostion of planktonic foraminifera: Experimental results and revised paleotemperature equations, Paleoceanography, 13, 150-160, 1998.

Bijma, J., Hemleben, C., Huber, B. T., Erlenkeuser, H., and Kroon, D.: Experimental determination of the ontogenetic stable isotope variability in two morphotypes of *Globigerinella siphonifera* (d'Orbigny), Mar. Micropaleontol., 35, 141-160, 1998.

Bijma, J., Hemleben, C., Oberhaensli, H., and Spindler, M.: The effects of increased water fertility on tropical spinose planktonic foraminifers in laboratory cultures, J. Foraminifer. Res., 22, 242-256, 1992.

Bijma, J., Pörtner, H. O., Yesson, C., and Rogers, A. D.: Climate change and the oceans - what does the future hold?, Mar Pollut Bull, 74, 495-505, 2013.

Branson, O., Kaczmarek, K., Redfern, S. A., Misra, S., Langer, G., Tyliszczak, T., Bijma, J., and Elderfield, H.: The coordination and distribution of B in foraminiferal calcite, Earth Planet Sci Lett, 416, 67-72, 2015.

Caron, D. A., Bé, A. W., and Anderson, O. R.: Effects of variations in light intensity on life processes of the planktonic foraminifer *Globigerinoides sacculifer* in laboratory culture, J. Mar. Biol. Assoc. U.K., 62, 435-451, 1982.

Catanzaro, E. J., Champion, C. E., Garner, E. L., Marinenko, G., Sappenfield, K. M., and Shields, W. R.: Boric acid: isotopic and assay standard reference materials, National Bureau of Standards, Institute for Materials Research, 1970.

Chmeleff, J., Horn, I., Steinhoefel, G., and von Blanckenburg, F.: In situ determination of precise stable Si isotope ratios by UV-femtosecond laser ablation high-resolution multi-collector ICP-MS, Chemical Geology, 249, 155-166, 2008.

Ciais, P., Sabine, C., Bala, G., Bopp, L., Brovkin, V., Canadell, J., Chhabra, A., DeFries, R., Galloway, J., Heimann, M., Jones, C., Le Quéré, C., Myneni, R. B., Piao, S., and Thornton, P.: Carbon and Other Biogeochemical Cycles. In: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, Stocker, T. F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S. K., Boschung, J., Nauels, A., Xia, Y., Bex, V., and Midgley, P. M. (Eds.), Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 2013.

Dickson, A.: An exact definition of total alkalinity and a procedure for the estimation of alkalinity and total inorganic carbon from titration data, Deep Sea Research Part A. Oceanographic Research Papers, 28, 609-623, 1981.

Dickson, A. G.: The development of the alkalinity concept in marine chemistry, Mar. Chem., 40, 49-63, 1992.
Dickson, A. G.: Thermodynamics of the dissociation of boric acid in synthetic seawater from 273.15 to 318.15 K, Deep Sea Research Part A. Oceanographic Research Papers, 37, 755-766, 1990.
Dickson, A. G., Sabine, C. L., Christian, J. R., and (Eds.): Guide to best practices for ocean CO_2 measurements, 191 pp., 2007.
DOE: Handbook of methods for the analysis of the various parameters of the cabon dioxide system in seawater; version 2, ORNL/CDIAC-74, 1994.
Fietzke, J., Heinemann, A., Taubner, I., Böhm, F., Erez, J., and Eisenhauer, A.: Boron isotope ratio determination in carbonates via LA-MC-ICP-MS using soda-lime glass standards as reference material, J Anal Atom Spectrom, 25, 1953-1957, 2010.
Foster, G., Pogge von Strandmann, P., and Rae, J.: Boron and magnesium isotopic composition of seawater, Geochemistry, Geophysics, Geosystems, 11, 2010.
Foster, G. L.: Seawater pH, pCO_2 and $[CO_3^{-2}]$ variations in the Caribbean Sea over the last 130 kyr: A boron isotope and B/Ca study of planktic foraminifera, Earth Planet Sci Lett, 271, 254-266, 2008.
Foster, G. L., Lear, C. H., and Rae, J. W. B.: The evolution of pCO_2, ice volume and climate during the middle Miocene, Earth Planet Sci Lett, 341, 243-254, 2012.
Foster, G. L., Ni, Y. Y., Haley, B., and Elliott, T.: Accurate and precise isotopic measurement of sub-nanogram sized samples of foraminiferal hosted boron by total evaporation NTIMS, Chemical Geology, 230, 161-174, 2006.
Foster, G. L. and Rae, J. W.: Reconstructing ocean pH with boron isotopes in foraminifera, Annu Rev Earth Planet Sci, 2015. 2015.
Gattuso, J.-P. and Hansson, L.: Ocean acidification: background and history. In: Ocean Acidification, Gattuso, J.-P. and Hansson, L. (Eds.), Oxford University Press, New York, NY, USA, 2011.
Gattuso, J.-P., Magnan, A., Billé, R., Cheung, W. W. L., Howes, E. L., Joos, F., Allemand, D., Bopp, L., Cooley, S. R., Eakin, C. M., Hoegh-Guldberg, O., Kelly, R. P., Pörtner, H.-O., Rogers, A. D., Baxter, J. M., Laffoley, D., Osborn, D., Rankovic, A., Rochette, J., Sumaila, U. R., Treyer, S., and Turley, C.: Contrasting futures for ocean and society from different anthropogenic CO_2 emissions scenarios, Science, 349, 2015.
Hemleben, C. and Bijma, J.: Foraminiferal population dynamics and stable carbon isotopes. In: Carbon Cycling in the Glacial Ocean: Constraints on the Ocean's Role in Global Change, Springer, 1994.
Hemleben, C., Spindler, M., Breitinger, I., and Ott, R.: Morphological and physiological responses ofGlobigerinoides sacculifer (Brady) under varying laboratory conditions, Mar. Micropaleontol., 12, 305-324, 1987.
Hemming, N. G., Guilderson, T. P., and Fairbanks, R. G.: Seasonal variations in the boron isotopic composition of coral: A productivity signal?, Global Biogeochem Cy, 12, 581-586, 1998.
Hemming, N. G. and Hanson, G. N.: Boron isotopic composition and concentration in modern marine carbonates, Geochim. Cosmochim. Acta, 56, 537-543, 1992.
Henehan, M. J., Foster, G. L., Bostock, H. C., Greenopa, R., Marshall, B. J., and Wilson, P. A.: A new boron isotope-pH calibration for *Orbulina universa*, with implications for understanding and accounting for 'vital effects', Earth Planet Sci Lett, in press. in press.
Henehan, M. J., Foster, G. L., Rae, J. W., Prentice, K. C., Erez, J., Bostock, H. C., Marshall, B. J., and Wilson, P. A.: Evaluating the utility of B/Ca ratios in planktic foraminifera as a proxy for the carbonate system: A case study of *Globigerinoides ruber*, Geochemistry, Geophysics, Geosystems, 16, 1052-1069, 2015.
Hergenröder, R., Samek, O., and Hommes, V.: Femtosecond laser ablation elemental mass spectrometry, Mass spectrometry reviews, 25, 551-572, 2006.
Hönisch, B., Allen, K., Hyams, O., Penman, D., Raitzsch, M., Ruprecht, J., Thomas, E., and Zachos, J.: Ocean acidification during the Cenozoic, Appl. Geochem., 26, S288-S288, 2011.
Hönisch, B., Bickert, T., and Hemming, N. G.: Modern and Pleistocene boron isotope composition of the benthic foraminifer *Cibicidoides wuellerstorfi*, Earth Planet Sci Lett, 272, 309-318, 2008.
Hönisch, B., Bijma, J., Russell, A. D., Spero, H. J., Palmer, M. R., Zeebe, R. E., and Eisenhauer, A.: The influence of symbiont photosynthesis on the boron isotopic composition of foraminifera shells, Mar. Micropaleontol., 49, 87-96, 2003.
Hönisch, B. and Hemming, N. G.: Surface ocean pH response to variations in pCO_2 through two full glacial cycles, Earth Planet Sci Lett, 236, 305-314, 2005.
Hönisch, B., Hemming, N. G., Archer, D., Siddall, M., and McManus, J. F.: Atmospheric carbon dioxide concentration across the mid-Pleistocene transition, Science, 324, 1551-1554, 2009.

Hönisch, B., Hemming, N. G., Grottoli, A. G., Amat, A., Hanson, G. N., and Bijma, J.: Assessing scleractinian corals as recorders for paleo-pH: Empirical calibration and vital effects, Geochim. Cosmochim. Acta, 68, 3675-3685, 2004.

Hönisch, B., Hemming, N. G., and Loose, B.: Comment on "A critical evaluation of the boron isotope-pH proxy: The accuracy of ancient ocean pH estimates" by M. Pagani, D. Lemarchand, A. Spivack and J. Gaillardet, Geochim. Cosmochim. Acta, 71, 1636-1641, 2007.

Hönisch, B., Ridgwell, A., Schmidt, D. N., Thomas, E., Gibbs, S. J., Sluijs, A., Zeebe, R. E., Kump, L., Martindale, R. C., Greene, S. E., Kiessling, W., Ries, J. B., Zachos, J. C., Royer, D. L., Barker, S., Marchitto Jr., T. M., Moyer, R., Pelejero, C., Ziveri, P., Foster, G. L., and Williams, B.: The geological record of ocean acidification, Science, 335, 1302-1302, 2012.

Hoppe, C. J. M., Langer, G., Rokitta, S. D., Wolf-Gladrow, D. A., and Rost, B.: Implications of observed inconsistencies in carbonate chemistry measurements for ocean acidification studies, Biogeosciences, 9, 2401-2405, 2012.

Horn, I., von Blanckenburg, F., Schoenberg, R., Steinhoefel, G., and Markl, G.: In situ iron isotope ratio determination using UV-femtosecond laser ablation with application to hydrothermal ore formation processes, Geochim. Cosmochim. Acta, 70, 3677-3688, 2006.

Jørgensen, B. B., Erez, J., Revsbech, N. P., and Cohen, Y.: Symbiotic photosynthesis in a planktonic foraminiferan, *Globigerinoides sacculifer* (Brady), studied with microelectrodes, Limnol Oceanogr, 30, 1253-1267, 1985.

Kaczmarek, K., Horn, I., Nehrke, G., and Bijma, J.: Simultaneous determination of δ^{11}B and B/Ca ratio in marine biogenic carbonates at nanogram level, Chemical Geology, 392, 32-42, 2015a.

Kaczmarek, K., Langer, G., Nehrke, G., Horn, I., Misra, S., Janse, M., and Bijma, J.: Boron incorporation in the foraminifer *Amphistegina lessonii* under a decoupled carbonate chemistry, Biogeosciences, 12, 1753-1763, 2015b.

Kaczmarek, K., Nehrke, G., Misra, S., Bijma, J., and Elderfield, H.: Investigating the effects of growth rate and temperature on the B/Ca ratio and δ^{11}B during inorganic calcite formation, Chemical Geology, 421, 81-92, 2016.

Kasemann, S. A., Meixner, A., Rocholl, A., Vennemann, T., Rosner, M., Schmidt, A. K., and Wiedenbeck, M.: Boron and oxygen isotope composition of certified reference materials NIST SRM 610/612 and reference materials JB-2 and JR-2, The Journal of Geostandards and Geoanalysis, 12, 405-416, 2001.

Katz, M. E., Cramer, B. S., Franzese, A., Hönisch, B., Miller, K. G., Rosenthal, Y., and Wright, J. D.: Traditional and emerging geochemical proxies in foraminifera, J. Foraminifer. Res., 40, 165-192, 2010.

Klochko, K., Cody, G. D., Tossell, J. A., Dera, P., and Kaufman, A. J.: Re-evaluating boron speciation in biogenic calcite and aragonite using 11B MAS NMR, Geochim. Cosmochim. Acta, 73, 1890-1900, 2009.

Klochko, K., Kaufman, A. J., Yao, W., Byrne, R. H., and Tossell, J. A.: Experimental measurement of boron isotope fractionation in seawater, Earth Planet Sci Lett, 248, 276-285, 2006.

Knoll, A. H. and Fischer, W. W.: Skeletons and ocean chemistry: the long view. In: Ocean Acidification, Gattuso, J.-P. and Hansson, L. (Eds.), Oxford University Press, New York, NY, USA, 2011.

Köhler-Rink, S. and Kuhl, M.: Microsensor studies of photosynthesis and respiration in the larger symbiont bearing foraminifera *Amphistegina lobifera*, and *Amphisorus hemprichii*, Ophelia, 55, 111-122, 2001.

Köhler-Rink, S. and Kühl, M.: Microsensor studies of photosynthesis and respiration in larger symbiotic foraminifera. I The physico-chemical microenviron-ment of *Marginopora vertebralis*, *Amphistegina lobifera* and *Amphisorus hemprichii*, Mar. Biol., 137, 473-486, 2000.

Lavigne, H. and Gattuso, J.-P.: Seacarb 1.2.3., an R package to calculate parameters of the seawater carbonate system, , 2013. online available at: http://cran.at.r-project.org/web/packages/seacarb/index.html, 2013.

Lazarov, M. and Horn, I.: Matrix and energy effects during in-situ determination of Cu isotope ratios by ultraviolet-femtosecond laser ablation multicollector inductively coupled plasma mass spectrometry, Spectrochimica Acta Part B: Atomic Spectroscopy, 111, 64-73, 2015.

le Roux, P. J., Shirey, S. B., Benton, L., Hauri, E. H., and Mock, T. D.: In situ, multiple-multiplier, laser ablation ICP-MS measurement of boron isotopic composition (delta B-11) at the nanogram level, Chemical Geology, 203, 123-138, 2004.

Lea, D., Bijma, J., Spero, H., and Archer, D.: Implications of a carbonate ion effect on shell carbon and oxygen isotopes for glacial ocean conditions. In: Use of Proxies in Paleoceanography, Springer, 1999.

Longerich, H. P., Jackson, S. E., and Günther, D.: Inter-laboratory note. Laser ablation inductively coupled plasma mass spectrometric transient signal data acquisition and analyte concentration calculation, J Anal Atom Spectrom, 11, 899-904, 1996.

Martínez-Botí, M., Foster, G., Chalk, T., Rohling, E., Sexton, P., Lunt, D., Pancost, R., Badger, M., and Schmidt, D.: Plio-Pleistocene climate sensitivity evaluated using high-resolution CO_2 records, Nature, 518, 49-54, 2015a.

Martínez-Botí, M., Marino, G., Foster, G., Ziveri, P., Henehan, M., Rae, J., Mortyn, P. G., and Vance, D.: Boron isotope evidence for oceanic carbon dioxide leakage during the last deglaciation, Nature, 518, 219-222, 2015b.

Mehrbach, C., Culberso.Ch, Hawley, J. E., and Pytkowic.Rm: Measurement of apparent dissociation-constants of carbonic-acid in seawater at atmospheric-pressure, Limnol. Oceanogr., 18, 897-907, 1973.

Misra, S., Owen, R., Kerr, J., Greaves, M., and Elderfield, H.: Determination of δ^{11}B by HR-ICP-MS from mass limited samples: Application to natural carbonates and water samples, Geochim. Cosmochim. Acta, 140, 531-552, 2014.

Oeser, M., Weyer, S., Horn, I., and Schuth, S.: High-Precision Fe and Mg Isotope Ratios of Silicate Reference Glasses Determined In Situ by Femtosecond LA-MC-ICP-MS and by Solution Nebulisation MC-ICP-MS, Geostand Geoanal Res, 38, 311-328, 2014.

Pagani, M., Lemarchand, D., Spivack, A., and Gaillardet, J.: A critical evaluation of the boron isotope-pH proxy: The accuracy of ancient ocean pH estimates, Geochim. Cosmochim. Acta, 69, 953-961, 2005.

Palmer, M. R., Pearson, P. N., and Cobb, S. J.: Reconstructing past ocean pH-depth profiles, Science, 282, 1468-1471, 1998.

Palmer, M. R., Spivack, A. J., and Edmond, J. M.: Temperature and pH controls over the fractionation during adsorption of boron on marine clay, Geochim. Cosmochim. Acta, 51, 2319-2323, 1987.

Pearson, P. N., Foster, G. L., and Wade, B. S.: Atmospheric carbon dioxide through the Eocene–Oligocene climate transition, Nature, 461, 1110-1113, 2009.

Pearson, P. N. and Palmer, M. R.: Estimating Paleogene atmospheric pCO_2 using boron isotope analysis of foraminifera, GFF, 122, 127-128, 2000.

Pearson, P. N. and Palmer, M. R.: Middle Eocene seawater pH and atmospheric carbon dioxide concentrations, Science, 284, 1824-1826, 1999.

R Core Team: R: A language and environment for statistical computing., R Foundation for Statistical Computing, Vienna, Austria, 2008.

Rae, J. W., Sarnthein, M., Foster, G. L., Ridgwell, A., Grootes, P. M., and Elliott, T.: Deep water formation in the North Pacific and deglacial CO_2 rise, Paleoceanography, 29, 645-667, 2014.

Rae, J. W. B., Foster, G. L., Schmidt, D. N., and Elliott, T.: Boron isotopes and B/Ca in benthic foraminifera: Proxies for the deep ocean carbonate system, Earth Planet Sci Lett, 302, 403-413, 2011.

Rhein, M., Rintoul, S. R., Aoki, S., Campos, E., Chambers, D., Feely, R. A., Gulev, S., Johnson, G. C., Josey, S. A., Kostianoy, A., Mauritzen, C., Roemmich, D., Talley, L. D., and Wang, F.: Observations: Ocean. In: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, Stocker, T. F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S. K., Boschung, J., Nauels, A., Xia, Y., Bex, V., and Midgley, P. M. (Eds.), Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 2013.

Rink, S., Kühl, M., Bijma, J., and Spero, H. J.: Microsensor studies of photosynthesis and respiration in the symbiotic foraminifer *Orbulina universa*, Mar. Biol., 131, 583-595, 1998.

Sadekov, A., Kerr, J., Langer, G., de la Fuente, M., Skinner, L., and Elderfield, H.: Understanding the mechanisms behind boron elemental and isotopic fractionation in the benthic foraminifera *Cibicidoides wuellerstorfi*, Poster, ICP12, Utrecht, 2016. 2016.

Salmon, K. H., Anand, P., Sexton, P. F., and Conte, M.: Calcification and growth processes in planktonic foraminifera complicate the use of B/Ca and U/Ca as carbonate chemistry proxies, Earth Planet Sci Lett, 2016. 2016.

Sanyal, A. and Bijma, J.: A comparitative study of northwest African and eastern equatorial Pacific upwelling zones as sources of CO_2 during glacial periods based on boron isotope paleo-pH estimation, Paleoceanography, 14, 753-759, 1999.

Sanyal, A., Bijma, J., Spero, H. J., and Lea, D. W.: Empirical relationship between pH and the boron isotopic composition of *Globigerinoides sacculifer*: Implications for the boron isotope paleo-pH proxy, Paleoceanography, 16, 515-519, 2001.

Sanyal, A., Hemming, N. G., Broecker, W. S., and Hanson, G. N.: Changes in pH in the eastern equatorial pacific across stage 5-6 boundary based on boron isotopes in foraminifera, Global Biogeochem Cy, 11, 125-133, 1997.

Sanyal, A., Hemming, N. G., Broecker, W. S., Lea, D. W., Spero, H. J., and Hanson, G. N.: Oceanic pH control on the boron isotopic composition of foraminifera: Evidence from culture experiments, Paleoceanography, 11, 513-517, 1996.
Sanyal, A., Hemming, N. G., Hanson, G. N., and Broecker, W. S.: Evidence for a higher pH in the glacial ocean from boron isotopes in foraminifera, Nature, 373, 234-236, 1995.
Sanyal, A., Nugent, M., Reeder, R. J., and Bijma, J.: Seawater pH control on the boron isotopic composition of calcite: Evidence from inorganic calcite precipitation experiments, Geochim. Cosmochim. Acta, 64, 1551-1555, 2000.
Schuessler, J. A. and von Blanckenburg, F.: Testing the limits of micro-scale analyses of Si stable isotopes by femtosecond laser ablation multicollector inductively coupled plasma mass spectrometry with application to rock weathering, Spectrochimica Acta Part B: Atomic Spectroscopy, 98, 1-18, 2014.
Seki, O., Foster, G. L., Schmidt, D. N., Mackensen, A., Kawamura, K., and Pancost, R. D.: Alkenone and boron-based Pliocene pCO_2 records, Earth Planet Sci Lett, 292, 201-211, 2010.
Spero, H. J. and Lea, D. W.: Intraspecific stable-isotope variability in the planktic foraminifera *Globigerinoides-sacculifer* - Results from laboratory experiments, Mar. Micropaleontol., 22, 221-234, 1993.
Spero, H. J. and Parker, S. L.: Photosynthesis in the symbiotic planktonic foraminifer *Orbulina universa*, and its potential contribution to oceanic primary productivity, The Journal of Foraminiferal Research, 15, 273-281, 1985.
Spivack, A. J. and Edmond, J. M.: Boron isotope exchange between seawater and the oceanic-crust, Geochim. Cosmochim. Acta, 51, 1033-1043, 1987.
Spivack, A. J., You, C.-F., and Smith, H. J.: Foraminiferal boron isotope ratios as a proxy for surface ocean pH over the past 21 Myr, Nature, 363, 149-151, 1993.
Uchikawa, J., Penman, D. E., Zachos, J. C., and Zeebe, R. E.: Experimental evidence for kinetic effects on B/Ca in synthetic calcite: Implications for potential $B(OH)_4^-$ and $B(OH)_3$ incorporation, Geochim. Cosmochim. Acta, 150, 171-191, 2015.
Van Andel, T. H.: Mesozoic/Cenozoic calcite compensation depth and the global distribution of calcareous sediments, Earth Planet Sci Lett, 26, 187-194, 1975.
Vengosh, A., Kolodny, Y., Starinski, A., Chivas, A. R., and McCulloch, M. T.: Coprecipitation and isotopic fractionation of boron in modern biogenic carbonates, Geochim. Cosmochim. Acta, 55, 2901-2910, 1991.
Vogl, J. and Rosner, M.: Production and certification of a unique set of isotope and delta reference materials for boron isotope determination in geochemical, environmental and industrial materials, Geostand Geoanal Res, 36, 161-175, 2012.
Wefer, G., Berger, W. H., Bijma, J., and Fischer, G.: Clues to ocean history: a brief overview of proxies. In: Use of proxies in paleoceanography: Examples from the South Atlantic, Fischer, G. and Wefer, G. (Eds.), Springer-Verlag, Berlin, Heidelberg, 1999.
Wolf-Gladrow, D. A., Bijma, J., and Zeebe, R. E.: Model simulation of the carbonate chemistry in the microenvironment of symbiont bearing foraminifera, Mar. Chem., 64, 181-198, 1999.
Wolf-Gladrow, D. A., Zeebe, R. E., Klaas, C., Körtzinger, A., and Dickson, A. G.: Total alkalinity: The explicit conservative expression and its application to biogeochemical processes, Mar. Chem., 106, 287-300, 2007.
Yu, J., Anderson, R., and Rohling, E.: Deep ocean carbonate chemistry and glacial-interglacial atmospheric CO_2 change, Oceanography, 27, 16-25, 2014.
Yu, J., Elderfield, H., and Hönisch, B.: B/Ca in planktonic foraminifera as a proxy for surface seawater pH, Paleoceanography, 22, PA2202, 2007.
Zeebe, R. E.: Vital effects in foraminifera do not compromise the use of $\delta^{11}B$ as a paleo-pH indicator: Evidence from modeling, Paleoceanography, 18, 2003.
Zeebe, R. E., Bijma, J., Hönisch, B., Sanyal, A., Spero, H. J., and Wolf-Gladrow, D. A.: Vital effects and beyond: a modelling perspective on developing palaeoceanographical proxy relationships in foraminifera. In: Biogeochemical Controls on Palaeoceanographic Environmental Proxies, Austin, W. E. N. and James, R. H. (Eds.), Geological Society of London, Special Publications, London, 2008.
Zeebe, R. E., Sanyal, A., Ortiz, J. D., and Wolf-Gladrow, D. A.: A theoretical study of the kinetics of the boric acid - borate equilibrium in seawater, Mar. Chem., 73, 113-124, 2001.
Zeebe, R. E. and Wolf-Gladrow, D. A.: CO_2 in Seawater: Equilibrium, Kinetics, Isotopes, Elsevier, Amsterdam, The Netherlands, 2001.

Tables and table legends

Ella Howes 11/11/2016 17:20

Deleted: ... [1]

Table 1: Average properties of the manipulated seawater culture medium from 4 samples (2 from the start of the incubation and two from the end of the incubation).

pH$_T$	***C*$_T$** **(µmol kg^{-1})**	***A*$_T$** **(µmol kg^{-1})**	***p*CO$_2$** **(µatm)**	**CO$_3^{2-}$** **(µmol kg^{-1})**	**HCO$_3^-$** **(µmol kg^{-1})**	**T** **(°C)**	**S**	**δ^{11}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

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

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

Table 3: Average (B/Ca) and average and median δ^{11}B values for the different experimental treatments.

pH$_T$	**CO$_3^{2-}$ (µmol kg^{-1})**	**Average δ^{11}B ± 2 s.e. (‰)**	**Median δ^{11}B ± 2 s.e. (‰)**	**B/Ca**	**N samples**
8.05 ± 0.02	238.7	17.8 ± 1.0	17.2 ± 1.0	1.27 (0.08)	9
8.05 ± 0.05	285.6	19.1 ± 0.7	18.8 ± 0.7	1.49 (0.06)	11
8.05 ± 0.03	533.9	20.0 ± 1.1	19.9 ± 1.1	0.77 0.03)	12
7.9 ± 0.02	296.6	16.8 ± 0.7	16.8 ± 0.7	0.92 (0.05)	18
7.7 ± 0.03	257.8	14.7 ± 0.8	14.9 ± 0.8	0.69 (0.04)	15

Figures and legends

Ella Howes 11/11/2016 17:20
Deleted: ... [2]

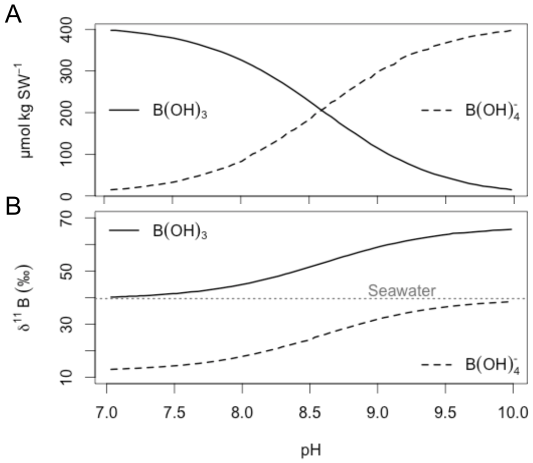

Fig 1: (A) Bjerrum plot showing the effect of pH on concentration of dissolved boron species at T = 25°C, S = 35 and $[B]_{total}$ 416 μmol kg^{-1}. (B) Effect of pH on boron isotopic composition of $B(OH)_4^-$ and $B(OH)_3$ with thermodynamic fractionation factor (α_{3-4}) = 1.0272 (Klochko et al.,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

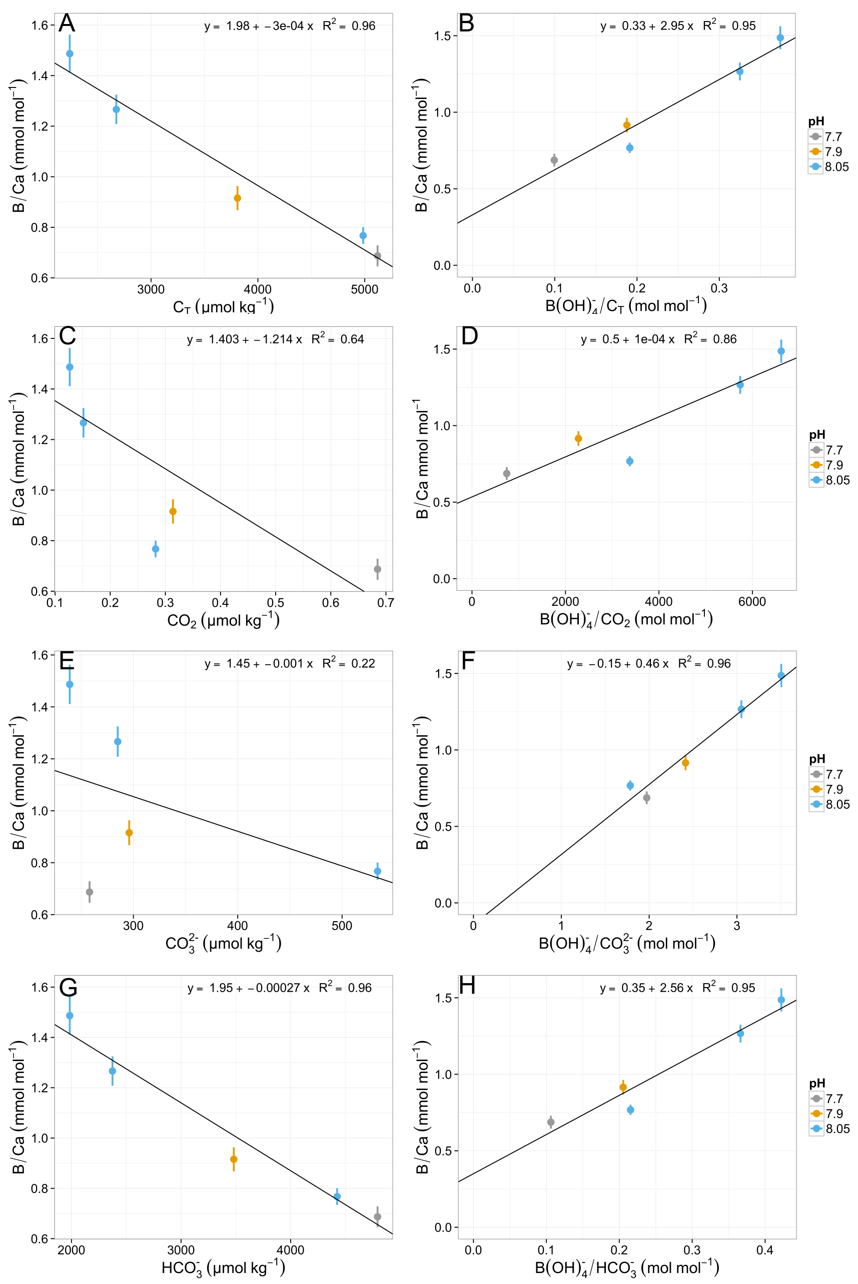

Fig. 2: B/Ca ratios plotted against (A) [C$_T$], (B) [B(OH)$_4^-$]/[C$_T$] ratio, (C) [CO$_2$], (D) [B(OH)$_4^-$]/[CO$_2$], (E) [CO$_3^{2-}$], (F) [B(OH)$_4^-$]/[CO$_3^{2-}$], (G) [HCO$_3^-$], (H) [B(OH)$_4^-$]/[HCO$_3^-$], error bars represent standard error.

Ella Howes 11/11/2016 17:20

Deleted:

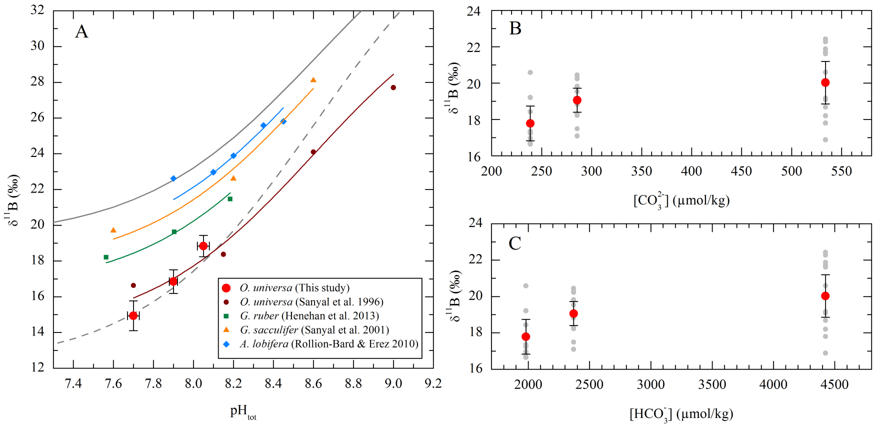

Fig 3: (A) Converted median $\delta^{11}B$ of cultured *O. universa* calcite (red circles) error bars represent ±2σ errors, solid grey line shows empirical values for seawater $\delta^{11}B_{borate}$ with a fractionation factor of $^{11\text{-}10}K_B$ = 1.020 (Hönisch et al., 2007) at T=23 °C and S=38. Dashed grey line shows the experimental $\delta^{11}B_{borate}$ curve with a fractionation factor of $^{11\text{-}10}K_B$ = 1.0272 (Klochko et al., 2006) at T=23 °C and S=38. (B) Median $\delta^{11}B$ cultured *O. universa* calcite grown at constant pH of 8.05 but varying $[CO_3^{2-}]$. (C) Median $\delta^{11}B$ of $B(OH)_4^-$ in cultured *O. universa* calcite grown at constant pH of 8.05 but varying $[HCO_3^-]$. Error bars represent ±2σ errors across all single laser ablation analyses per treatment.

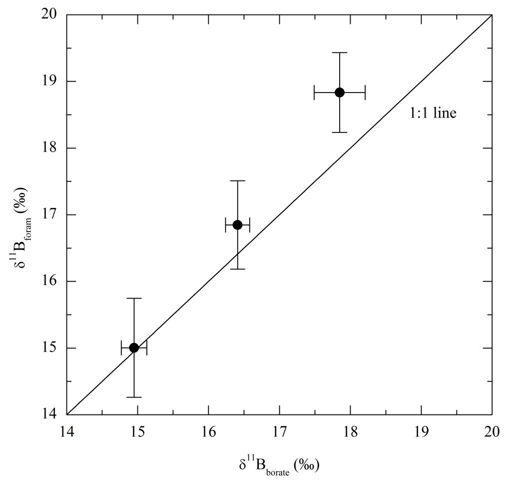

Fig. 4: $\delta^{11}B$ of borate versus that of calcite. $\delta^{11}B_{borate}$ was calculated using the fractionation factor of $^{11\text{-}10}K_B = 1.0272$ (Klochko et al., 2006) at T=23 °C and S=38. Error bars for $\delta^{11}B_{foram}$ represent $\pm 2\sigma$ errors, and for $\delta^{11}B_{borate}$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