1 Decoupled carbonate chemistry controls on the incorporation of boron

2 into *Orbulina universa*.

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21 **Abstract**

22 In order to fully constrain paleo-carbonate systems, proxies for two out of seven

23 parameters, plus temperature and salinity are required. The boron isotopic

24 composition ($\delta^{11}B$) of planktonic foraminifera shells is a powerful tool to reconstruct

25 changes in past surface ocean pH. As $B(OH)_4^-$ is substituted into the biogenic calcite

26 lattice in place of CO_3^{2-}, it has been suggested that B/Ca ratios in biogenic calcite are

27 a possible proxy for $[CO_3^{2-}]$. However, differentiating between the effects of pH and

28 $[CO_3^{2-}]$ is problematic, as they co-vary closely in natural systems, and so the major

29 control on boron incorporation remains unclear. To deconvolve the effects of pH and

30 $[CO_3^{2-}]$ on the B/Ca ratio and to test whether $\delta^{11}B$ remains constant at constant pH,

31 but under changing $[CO_3^{2-}]$ (pH 8.05 with 238, 285 and 532 µmol kg^{-1} CO_3^{2-}) and

32 vice versa, we decoupled pH and $[CO_3^{2-}]$ (276 ± 19.5 µmol kg^{-1} CO_3^{2-} with pH 7.7,

33 7.9 and 8.05) and grew the planktonic foraminifer *Orbulina universa* in these

34 manipulated culture media. Measurements of the isotope composition of boron and

35 the B/Ca ratio were performed simultaneously using a femtosecond laser ablation

36 system coupled to an MC ICP-MS. Results show that $\delta^{11}B$ is controlled by pH and

37 does not respond to changes in $[CO_3^{2-}]$. On the other hand, the B/Ca ratio is driven by

38 $[HCO_3^-]$ independently of pH. This suggests that B/Ca ratios in foraminiferal calcite

39 may be used as a second, independent, proxy for paleo-carbonate system

40 reconstructions.

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42
43
44 **Introduction**
45
46 Before the Anthropocene, the atmospheric CO_2 concentration was governed by the

47 surface ocean $[CO_2]$, simply because the carbon content of the ocean is 65 times

48 larger than that of the atmosphere (Siegenthaler and Sarmiento, 1993). Hence,

49 understanding the global carbon cycle and the evolution of atmospheric pCO_2 in Earth

50 history requires knowledge of the dynamics of the oceanic carbonate chemistry. The

51 unprecedented magnitude and rate of carbon emissions since the industrial revolution

52 has resulted in warming, and acidification of the ocean (Bijma et al., 2013; Ciais et

53 al., 2013; Gattuso and Hansson, 2011; Rhein et al., 2013). As a result, the interest in

54 the reconstruction of seawater carbonate chemistry to identify ocean acidification in

55 Earth history experienced another impetus (Hönisch et al., 2012; Martínez-Botí et al.,

56 2015). The reconstruction of the full oceanic carbonate chemistry requires proxies of

57 at least two independent parameters of the carbonate system, in addition to

58 temperature and salinity.

59

60 The most promising tool, for reconstructing pH is the boron isotopic composition

61 $(\delta^{11}B)$ of biogenic carbonate producers such as foraminifera and corals (Hönisch et

62 al., 2004; Rae et al., 2011; Sanyal et al., 2001; Sanyal et al., 1996). Boron exists in

63 seawater primarily in the form of two species, boric acid $(B(OH)_3)$ and borate ion

64 $(B(OH)_4^-;$ Fig. 1A). As for all weak acids, the relative abundance between these two

65 species is controlled by pH (Dickson, 1990; DOE, 1994) . Because of the isotopic

66 fractionation between the two aqueous species (Fig 1B; $\alpha_{4-3} = R_{B(OH)4^-}/R_{B(OH)3}$), the

67 boron isotopic composition of each species is also pH dependent (Hemming and

68 Hanson, 1992; Palmer et al., 1987; Sanyal et al., 1996; Sanyal et al., 2000). At low

69 pH (<7) nearly all boron is present in the form of boric acid, whereas at high pH (>10)

70 boron primarily exists as borate. $B(OH)_3$ is enriched in the stable isotope ^{11}B

71 compared to $B(OH)_4^-$, with a constant isotopic fractionation of 27.2 ‰ between the

72 two boron species (Klochko et al., 2009; Klochko et al., 2006). Consequently, as the

73 relative concentration of the dissolved species changes with pH, so does their isotopic

74 composition. Because it is assumed that only the charged species, borate, is

75 incorporated into the calcite lattice (Hemming and Hanson, 1992; Vengosh et al.,

76 1991) the boron isotopic composition of marine carbonates thus records the pH that

77 prevailed when the calcium carbonate was precipitated.

78

79 The $\delta^{11}B$ of marine calcifiers such as corals, bivalves, foraminifera, pteropods, etc.

80 can be used as a proxy for paleo pH and therefore provide clues on the evolution of a

81 key parameter of the oceanic carbonate chemistry. For instance, Spivack et al. (1993)

82 estimated that the pH of the surface ocean may have changed from 7.4 in the Miocene

83 to 8.2 in the Holocene. Sanyal et al. (1995) estimated that during the last glacial

84 maximum the deep ocean pH was about 0.3 pH unit higher and the surface ocean pH

85 about 0.2 pH unit higher compared to that of the modern values. Inspired by such

86 promising results several groups took on the challenge to reconstruct ocean pH. In the

87 subsequent years, many studies have dealt with reconstructing past oceanic pH across

88 different time scales using the boron isotopic composition of mainly foraminifera but

89 also corals (Foster et al., 2012; Foster et al., 2006; Hemming et al., 1998; Hönisch et

90 al., 2011; Hönisch et al., 2008; Hönisch and Hemming, 2005; Hönisch et al., 2009;

91 Hönisch et al., 2007; Hönisch et al., 2012; Martínez-Botí et al., 2015; Palmer et al.,

92 1998; Pearson and Palmer, 2000; Pearson and Palmer, 1999; Sanyal et al., 1997;

93 Sanyal et al., 1995; Spivack et al., 1993).

94

95 $\delta^{11}B$ is proved to be a reliable recorder of pH but, in the aforementioned

96 reconstructions, the second carbonate system parameter used to reconstruct pCO$_2$ was

97 based on assumptions. In existing boron-based reconstructions, investigators

98 attempted to estimate total alkalinity (A_T) or $[CO_3^{2-}]$ from modern ocean conditions or

99 reconstructions of carbonate compensation depth (CCD). In the modern ocean A_T is

100 linearly correlated with salinity, because total alkalinity is the charge balance between

101   the major conservative ions in seawater, and this charge balance varies with salinity

102   (Dickson, 1981; Dickson, 1992; Wolf-Gladrow et al., 1999; Wolf-Gladrow et al.,

103   2007). Therefore, total alkalinity is a conservative parameter. It is assumed that the

104   modern salinity–A_T relationship was constant over time so that A_T can be estimated

105   from reconstructions of salinity using sea-level records (Foster, 2008; Hönisch et al.,

106   2009). However, salinity and alkalinity may be decoupled in space and time through

107   weathering and changes in the hydrological cycle and, reliable proxies for regional

108   salinity reconstructions are not available to date. Another approach is based on the

109   assumption that seawater $[Ca^{2+}]$ has remained proportional to A_T over time so that A_T

110   can be adjusted in a way that the water column is exactly saturated with respect to

111   calcite at the lysocline (~500 m above the CCD; Pearson and Palmer, 2000). In other

112   studies, $[CO_3^{2-}]$ was estimated through adjusting the surface ocean $[CO_3^{2-}]$ to match

113   the CCD. The CCD, however, is not uniform through space and time (Van Andel,

114   1975), calling into question these approaches for estimating past A_T.

115

116   Considering the relatively large uncertainties afflicted with the constraints described

117   above, it is of utmost importance to develop proxies for a carbonate system parameter

118   in addition to pH. A potential one is the B/Ca ratio of planktonic foraminifera that has

119   been used as a proxy for estimating past changes in $[CO_3^{2-}]$ (Foster, 2008), given that

120   the concentration of borate $B(OH)_4^-$) increases with pH and pH co-varies with $[CO_3^{2-}$

121   ]. However, it is challenging, if not impossible, to identify the parameter controlling

122   B/Ca based on samples that have grown in natural seawater because pH and carbonate

123   chemistry parameters co-vary closely in natural systems. To disentangle their effects

124   on a proxy it is necessary to deconvolve the carbonate chemistry.

125

126   A more recent study (Allen et al., 2012) has shown that the B/Ca ratio of planktonic

127   foraminifera also decreases with increasing total inorganic carbon (C_T) or $[HCO_3^-]$) at

128   constant pH (i.e. $[B(OH)_4^-]$ was constant while $[CO_3^{2-}]$ and $[HCO_3^-]$ were increased),

129   suggesting that borate and carbon species compete for the inclusion into the calcite

130   lattice. However, in this study pH was not decoupled from $[CO_3^{2-}]$, leaving the

131   question open whether the B/Ca ratio in planktonic foraminifera is influenced by the

132   ratio between $[B(OH)_4^-]$ and C_T or $[HCO_3^-]$. Kaczmarek et al. (2015b) decoupled the

133   carbonate chemistry and showed that B/Ca in the benthic foraminifer *Amphistegina*

134   *lessonii* is influenced by the ratio between $[B(OH)_4^-]$ and $[HCO_3^-]$, rather than by pH

135   or $[CO_3^{2-}]$. Recently, Henehan et al. (2015) suggested that B/Ca in *Globigerinoides*

136   *ruber* might be controlled by $[PO_4]$. We believe that this relationship results from a

137   co-variation between ocean carbonate chemistry and nutrients because respiration of

138   organic matter will release both carbon and nutrients. Here we conducted experiments

139   with a planktonic foraminifer and decoupled pH and $[CO_3^{2-}]$, as performed by

140   Kaczmarek et al. (2015b). We also show that combined measurements of $\delta^{11}B_{calcite}$

141   and B/Ca of the same species as conducted in our study might be used to fully

142   constrain the carbonate chemistry in Earth history.

143

144

145   **Methods**

146   *Collection and culturing:*

147   Living specimens of *Orbulina universa* were collected daily using a 57 cm diameter

148   WP2 plankton net (200 µm mesh size), between July and September 2012 at Point B,

149   Villefranche-sur-Mer, France (43.41˚N, 7.19˚E) and maintained until gametogenesis

150   in laboratory cultures at the Laboratoire d'Oceanographie de Villefranche.

151   Established procedures for maintaining planktonic foraminifera in laboratory culture

152   were used (Bemis et al., 1998; Bijma et al., 1998; Spero and Lea, 1993). Briefly,

153   specimens were identified, measured with a light microscope, and transferred to 0.2

154   µm-filtered seawater, whose carbonate chemistry was accurately determined and

155   subsequently modified. Specimens were maintained individually in air-tight 100 ml

156   acid-washed SCHOTT DURAN® bottles that were sealed without an air space and

157   placed upside down into thermostated water baths maintained at a temperature of

158   23°C (±0.2°C). Light was provided by 4, 39 W fluorescent tubes (JBL Solar Ultra

159   Marin Day), with reflectors, (at a distance of ca. 15 cm from the water surface), with a

160   12:12 h L: D photoperiod. The average irradiance, measured with a LI-193 sensor

161   (LiCOR) in the culture jars was about 290 µmol photons $m^{-2} s^{-1}$.

162

163   The foraminifers were fed a one-day-old brine shrimp *Artemia nauplius* every second

164   day until gametogenesis. The brine shrimps were hatched in modified seawater from

165   the same batch as used for culturing the foraminifera. Just prior to feeding, hatched

166   nauplii were transferred once again to fresh medium from the same batch. After

167   feeding culture jars were topped up with medium from the same batch to prevent the

168   formation of a headspace. Empty shells were collected within 24 h after successful

169   gametogenesis, rinsed in deionised water and archived in covered micro paleo-slides

170   for later analysis. Approximately 35 tests were grown for each experimental

171   treatment. Culture water samples were collected at the start and end of the

172   experiments to verify the boron concentration, its isotopic composition and the

173   carbonate system parameters.

174

175   *Modified seawater chemistry*

176   The objective of these experiments was to decouple seawater pH and $[CO_3^{2-}]$ and

177   create treatments with a constant pH and varying carbonate ion concentration and

178   treatments with a constant carbonate ion concentration but varying pH. To decouple

179   the effects of pH_T and $[CO_3^{2-}]$, seawater carbonate chemistry was modified

180   manipulating pH_T, using NaOH and HCl, and dissolved inorganic carbon (C_T) by

181   adding gravimetrically carbonate and bicarbonate or bubbling with CO_2. Calculations

182   were made using csys_vari.m (Zeebe et al., 2001) with carbonic acid dissociation

183   constants of (Mehrbach et al., 1973). Temperature (23^oC) and salinity (38.0) were

184   kept constant (Table 1).

185

186   To enable single shell analysis by LA-MC-ICP-MS, the boron concentration was

187   increased to about 10 times the concentration of natural seawater by adding boric acid

188   to the culture water (see Sanyal et al., 2001; Sanyal et al., 1996; Sanyal et al., 2000).

189   The pH_T and C_T were then modified via titration with boron free NaOH (1N) and HCl

190   (1N) to bring the experimental pH to desired levels of 7.70 ± 0.03, 7.90 ± 0.02 and 8.

191   05 ± 0.05, respectively. Culture water samples collected at the start and at the end of

192   each experiment showed that pH remained nearly constant throughout each

193   experiment. The boron isotopic composition of each culture treatment is provided in

194   Table 1. pH of the culture solutions was measured using a Metrohm, 826 mobile pH

195   meter with glass electrode (Metrohm, electrode plus) calibrated to the total scale using

196   TRIS and 2-aminopyridine buffer solutions (Dickson et al., 2007) adjusted to a

197   salinity of 38.0 (Martinez-Boti et al. 2015). Total alkalinity (A_T) samples (150 mL)

198   were filtered on GF/F and measured potentiometrically using a Metrohm Tritando 80

199   titrator and a Metrohm, electrode plus glass electrode (Dickson et al., 2007). 60 ml

200   samples was also taken at the start and end of incubations and poisoned with 10 μL of

201   saturated $HgCl_2$ pending determination of dissolved inorganic carbon (C_T). Samples

202   were measured using an AIRICA (Marianda, Kiel) fitted with a Licor 6262 infra-red

203   gas analyser. All parameters of the carbonate system were calculated from A_T and pH_T

204   (Hoppe et al., 2012) using the R package seacarb (Lavigne and Gattuso, 2013).

205

206

Analysis of O. universa

208   For simultaneous determination of the B isotopic composition and its concentration a

209   Fiber Optics Spectrometer (Maya2000 Pro, Ocean Optics) was connected to the torch

210   of a Thermo Finnigan Neptune multiple-collector inductively coupled plasma mass

211   spectrometer (MC-ICP-MS) at the Leibniz University of Hannover. Laser ablation on

212   reference material and samples was performed by an in-house build UV-femtosecond

213   laser ablation system based on a regenerative one box femtosecond laser (Solstice

214   Newport/Spectra Physics). A detailed description of the method used for the

215   simultaneous determination of B concentration and $\delta^{11}B$ of *O.universa* can be found

216   in Kaczmarek et al. (2015a). A summary of the procedure is given below.

217

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

219   The B intensity of a reference material corresponds to its known B concentration.

220   Based on this relationship the unknown B concentration of a sample can be

221   calculated. However, our measurements of the reference material (NIST SRM 610)

222   and samples were not performed at the same laser repetition rate hence their B ratio is

223   not proportional. The correction for different laser repetition rates was realized by the

224   analysis of calcium in the reference material and in the sample because their Ca

225   concentrations are known (NIST SRM 610: 8.45%, $CaCO_3$: 40%) using an optical

226   spectrometer (Maya2000 Pro). More information on this procedure is provided by

227   Longerich et al. (1996).

228

Calcium analysis

230   The Maya2000 Pro is a high-sensitivity fiber optical spectrometer. It has a measuring

231   range between 250 to 460 nm with a resolution of 0.11 nm covering the first order

232   emission lines of Mg II, Ca II, Sr II Ba II and Li II. It is equipped with a back-thinned

233   2D FFT-CCD detector, and a grating with a groove density of 1200 lines/mm. The

234   optical fiber used is two meters long (attenuation of the photon flux is length

235   dependent) connecting the spectrometer with the coupling lens at the end of the

236   plasma torch of the MC-ICP-MS (Thermo Finnigan Neptune). Ca II ion lines were

237   measured at a wavelength of 393.48 and 396.86 nm. At these wavelengths the Ca

238   spectra shows no detectable interferences for the matrices used. The acquisition

239   parameters were set to acquire 220 cycles per analysis with an integration time of 1 s

240   for each cycle. Because of the stable Background (BG) signal detected for the first 40

241   cycles, BG correction was done by subtracting its intensity from the intensity of the

242   reference and the sample material.

243

244   *Boron Isotope Analysis - 194 nm femtosecond laser ablation*

245   The in-house built laser ablation system is based on a 100 femtosecond Ti-sapphire

246   regenerative amplifier system operating at a fundamental wavelength of 777nm in the

247   infrared spectrum. Subsequent harmonic generations produce the wavelengths 389 nm

248   in the second, 259 nm in the third and 194 nm in the fourth harmonic. The pulse

249   energies measured with a pyroelectric sensor (Molectron, USA) are 3.2mJ/pulse at

250   777nm, 0.7 mJ/pulse at 259 nm, and 0.085 mJ/pulse at 194 nm. After the fourth

251   harmonic generation stage, the 194 nm beam is steered by eight dichronic mirrors into

252   a 8x objective (NewWave-Research, USA) and focussed onto the sample. Spot size

253   was set to 50 µm for the reference material and the samples. Within this spot an

254   energy density of ~2 J/cm^2 is maintained. Reference material measurements were

255   performed in raster mode (100µm x 100µm) at 10 Hz, samples were ablated at 8-50

256   Hz depending on B concentration.

257

258   *Boron Isotope Analysis - Acquisition parameters*

259   All measurements are carried out in low mass resolution (Δm/m=350 where m is the

260   mass of the ion of interest and Δm is the mass difference between its 5 and 95% peak

261   height). Compact discrete dynode multipliers (CDD, Thermo) are attached to faraday

262   cups at the low site on L4 and the high site on H4. The low resolution mode is

263   sufficient enough to resolve potential interferences from doubly charged ions due to

264   the intrinsic high resolution in the low mass region. Possible interferences are the

265   clusters of $^{40}Ar^{4+}$ or $^{20}Ne^{2+}$ which are well resolved to the background level. Prior to

266   each analytical session the instrument was tuned for optimal peak shape. Instrumental

267   operating conditions are reported in Table 2. All measurements were performed at

268   plateau voltage of the CDDs, which was checked prior to every analytical session.

269   Before the beginning of sample analysis, measurements of NIST SRM 610 were

270   continued until instrumental drift (due to warm-up) was less than 200 ppm over a

271   bracketing sequence duration of twelve minutes. Boron signal intensities of NIST

272   SRM 610 and samples were matched within 10% in signal intensity by adapting the

273   laser repetition rate. The acquisition parameters in static mode for analysis of NIST

274   SRM 610 and samples were set to acquire 200 cycles of 1 s integrations each. During

275   the first 40 cycles, the background signal was acquired whereas the remaining cycles

276   represent the sum of the background and the reference material, or the background

277   and the sample signals. A complete measurement consisting of 200 cycles of a single

278   reference material/sample took four minutes before the next sample was introduced.

279   For analysis we adopted the standard sample bracketing procedure and the B isotopic

280   composition is reported using the delta notation:

281

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

283

284   Where NIST 610-1 and NIST 610+1 refer to the analysis of the reference material

285   before and after the sample. The uncertainty of the samples was calculated according

286   to:

287

288    $$2SE_{\delta^{11}B_{sample}}(‰) =$$

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

290

291   Where $^{11/10}$B ratios represent mean values of the reference material and the sample

292   calculated from one measurement, respectively (based on 160 cycles) and SE

293   represents the standard error of the $^{11/10}$B ratios. Due to the natural inhomogeneity of

294   the samples the analytical uncertainty is represented best by repeated measurements

295   of the homogenous reference material given by:

296

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

298

299 Where the measurements of the $(^{11/10}B)_{-1}$ and $(^{11/10}B)_{+1}$ ratios of NIST 610 were

300 performed before and after the measurement of $(^{11/10}B)_0$, respectively. For the

301 determination of the analytical uncertainty and external reproducibility all

302 measurements of NIST 610 performed between each sample measurement were taken

303 into account. On average the analytical uncertainty and external reproducibility is

304 0.66‰.

305

306 *Conversion of $\delta^{11}B_{O.universa}$ to seawater scale*

307 Due to the B addition to our culture media, the $\delta^{11}B_{seawater}$ shifted from 37.95 to on

308 average 4.66 ‰. Therefore, the $\delta^{11}B_{O.universa}$ shifted accordingly. In order to compare

309 our *O. universa* data to published values (Fig. 3A), the measured $\delta^{11}B$ was converted

310 to seawater scale using (Zeebe & Wolf-Gladrow, 2001):

311

312
$$\delta^{11}B_c = \alpha_{sw-msw} \times \delta^{11}B_m + \varepsilon \quad \text{Eq. (4)}$$

313

314 Where ε is (α_{sw-msw} -1) x 1000, $\delta^{11}B_c$ represents the converted $\delta^{11}B$ for the measured

315 value ($\delta^{11}B_m$), α_{sw-msw} is a fractionation factor expressing the difference between the

316 natural seawater and manipulated seawater:

317

318
$$\alpha_{sw-msw} = (\delta^{11}B_{sw} + 10^3)/(\delta^{11}B_{msw} + 10^3) \quad \text{Eq. (5)}$$

319

320 *Statistics:*

321 Lamtool was used for analysis and background correction of the $\delta^{11}B$ data. All other

322 statistics were carried out using R (R Core Team, 2008). Error bars represent ± 2σ

323 errors, correlations were calculated by linear regression.

324

325 **Results:**

326 *B/Ca ratios:*

327   The B/Ca ratio of *O. universa* shows a strong negative correlation ($R^2 = 0.96$) with C_T

328   irrespective of the pH_T of the culture media (Fig 2A). It is also correlated to $[CO_2]$ but

329   to a lesser extent ($R^2 = 0.64$; Fig 2C). B/Ca also decreases with increasing $[CO_3^{2-}]$ in

330   specimens grown under a pH_T of 8.05, (Fig 2E). However, the B/Ca ratio of

331   specimens grown under lower pH_T values (7.9 and 7.7) is negatively offset from the

332   relationship found at pH_T 8.05 and the overall correlation of B/Ca and $[CO_3^{2-}]$ is very

333   low ($R^2 = 0.2$; Fig 2E). Of all the carbonate species, the B/Ca ratio exhibits the best,

334   negative, relationship with increasing $[HCO_3^-]$, irrespective of the pH_T of the culture

335   medium ($R^2 = 0.96$; Fig 2G). Plotted against the ratio of $[B(OH)_4^-]$ over each of the

336   carbon species (Fig 2B, D, F, H), the correlations are high for all combinations but

337   highest for $[B(OH)_4^-]/[CO_3^{-2}]$.

338

339

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

341   Single, measured $\delta^{11}B$ values of *O. universa* are given in Supplementary Table 1,

342   errors are calculated according to Eq. (2). Mean and converted values using Eq. (4)

343   and Eq. (5) are shown in Fig. 3A and Table 3. The fractionation of boron isotopes in

344   the shells of *O. universa* is dependent on the pH of the culture medium, increasing

345   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

346   fractionation curve of $B(OH)_4^-$ obtained for artificial seawater by (Fig 3A; Klochko et

347   al., 2006). $\delta^{11}B$ increases slightly with increasing $[CO_3^{2-}]$ at constant pH_T (Fig 3B).

348   However, the data are all within analytical error, suggesting that there is no significant

349   effect of $[CO_3^{2-}]$ on $\delta^{11}B$.

350

351

352

353   **Discussion:**

354   *B/Ca:*

355   Foster (2008) identified $[CO_3^{2-}]$ as having a major control on B/Ca in samples of

356   foraminifera from down core samples and core tops. A similar conclusion was

357   reached by Allen et al. (2011) for *O. universa*. These authors demonstrated a trend of

358   decreasing B/Ca with increasing pH and $[CO_3^{2-}]$; however, due to the co-variations of

359   the carbonate system in natural seawater it is difficult to identify the differential

360   effects of the individual parameters. Experimentally decoupling pH_T from other

361   parameters of the carbonate system using modified seawater media allows us to

362   decouple the relationships and identify the controlling carbon species. Our results

363   demonstrate that the amount of boron incorporated into *O. universa* calcite is a

364   function of C_T (Fig. 2A). As C_T increases, B/Ca decreases, suggesting that $B(OH)_4^-$

365   competes with carbon species for inclusion into the calcite lattice. When B/Ca ratios

366   are plotted against $[CO_2]$, the relationship is similar to that of C_T, however only <1%

367   of C_T is in the form of CO_2 so this species is unlikely to have a major control on

368   boron incorporation. The remaining >99% is ~10% CO_3^{2-} and ~ 90% HCO_3^- (Zeebe

369   and Wolf-Gladrow, 2001). Due to the strong correlation of the B/Ca ratio and

370    $[B(OH)_4^-]/[$ $C_T]$, one could argue that foraminifera utilize both HCO_3^- and CO_3^{2-} as

371   substrate for calcification and, therefore, that C_T is the factor controlling the B/Ca

372   ratios. However, because $[HCO_3^-]$ and $[CO_3^{2-}]$ in our treatments, increase and

373   decrease with decreasing pH_T, respectively (Table 1), we can distinguish between

374   bicarbonate and carbonate ion control over the B/Ca ratio.

375

376   At constant pH_T, the relationship between B/Ca and $[CO_3^{2-}]$ (Fig. 5.1C) supports the

377   hypothesis of competition between CO_3^{2-} and $B(OH)_4^-$. However, when $[CO_3^{2-}]$ is

378   held constant and pH_T is decreased, B/Ca significantly decreases despite the fact that

379    $[CO_3^{2-}]$ remains more or less constant (Fig 2 E, Table 1). If the same relationships are

380   examined for B/Ca and $[HCO_3^-]$ a strong correlation between $[HCO_3^-]$ and B/Ca is

381   observed for both, the absolute concentration of HCO_3^- (Fig. 2G) and also for the ratio

382   of $[B(OH)_4^-]/[HCO_3^-]$ with no effect of changing pH_T (Fig. 2H). The close correlation

383   between $[CO_3^{2-}]$ and B/Ca at constant pH_T can be explained by the corresponding

384   increases in $[HCO_3^-]$ in these treatments (Table 1).

385

386   In agreement with our results, the study of Allen et al. (2012) investigated the effects

387   of decoupling pH and the carbonate system on B/Ca and suggest that $B(OH)_4^-$

388   competes with carbon species for inclusion into the calcite lattice in three planktonic

389   species *Globigerinoides sacculifer*, *Globigerinoides ruber*, and *Orbulina universa*.

390   However, analysis of planktonic foraminifera from core tops revealed correlation

391   between B/Ca and exclusively $B(OH)_4^-/HCO_3^-$ (excluding $B(OH)_4^-/CO_3^{2-}$and $B(OH)_4^-$

392    $/C_T$.) Yu et al., (2007). A recent study by Kaczmarek et al. (2015b) shows the same

393 competition between $B(OH)_4^-$ and HCO_3^- in the benthic species *Amphistegina*

394 *lessonii* cultured in a pH-$[CO_3^{2-}]$ decoupled seawater. The observation that B/Ca is

395 driven by $B(OH)_4^-/HCO_3^-$ and not related to CO_3^{2-} only becomes visible at higher pH

396 (8.6) when $[B(OH)_4^-]$ is sufficiently high (see Fig. 6 and Table S1 in Kaczmarek et al.,

397 2015b). Below 8.6 foraminiferal B/Ca also correlates with $B(OH)_4^-/CO_3^{2-}$.

398

399 The finding that $B(OH)_4^-/HCO_3^-$ is controlling on boron incorporation in *O. universa*

400 calcite is also in agreement with the hypotheses of Hemming and Hanson (1992) who

401 suggested that only $B(OH)_4^-$ is incorporated into marine carbonates with the partition

402 coefficient defined below.

403

404 $$K_D = \frac{[B/Ca]_{solid}}{[B(OH)_4^- / HCO_3^-]_{seawater}} \qquad \text{Eq. (6)}$$

405

406 To summarize, based on our study, we can eliminate a control by $[CO_3^{2-}]$ but cannot

407 exclude $[B(OH)_4^-/CO_3^{2-}]$. By comparison to the B/Ca control in the benthic

408 foraminifer *Amphistegina lessonii* (Kaczmarek et al., 2015), we assume B/Ca in

409 planktonic foraminifera is also a function of $[B(OH)_4^-/HCO_3^-]$.

410

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

412 As the various species of inorganic carbon and pH_T are tightly linked, it is still to be

413 experimentally demonstrated, beyond doubt, whether only pH_T and/or the

414 concentration of one or several carbonate species might have an effect on $\delta^{11}B$. The

415 results for treatments with varying pH_T and constant carbonate ion concentration

416 displayed the same relationship as those from the calibration curve for *O. universa*

417 produced by Sanyal et al. (1996) but the absolute values for a given pH_T are slightly

418 lower by approximately 1 to 2‰ when compared to the values corrected to the

419 fractionation factor suggested by Klochko et al. (2006) (Zeebe et al., 2008). The

420 effects of the unnaturally high C_T and A_T values in the treatments cannot be

421 discounted as the cause of this difference, although it is unlikely because the $\delta^{11}B$

422 values proved to be robust when a range of $[CO_3^{2-}]$ was used. The values produced for

423 $\delta^{11}B$ in this study match closely with the values for artificial seawater given by

424 Klochko et al. (2006). This is caused by the suppression of the vital effects imposed

425   by *O. universa*. Theoretical considerations demonstrate that at 10X boron

426   concentration compared to natural seawater, vital effects are suppressed and the

427   isotopic value of biogenic calcite approaches the value of the borate species being

428   taken up (Zeebe, 2003). This was confirmed by the comparison of the boron isotopic

429   values of *O. universa* grown at low and high light (Hönisch et al., 2003) and supports

430   the notion that borate is indeed the species being taken up. There is no effect of

431   varying $[CO_3^{2-}]$ on $\delta^{11}B$ of samples grown at the same pH but, most importantly, in

432   light of the results obtained for the B/Ca ratio, there is also no effect of $[HCO_3^-]$ (Fig.

433   3C).

434

435

436   *Proxy implications:*

437   Based on our results and other culture studies, it becomes clear that despite strong

438   biological effects on the ambient carbonate chemistry (Köhler-Rink and Kuhl, 2001;

439   Köhler-Rink and Kühl, 2000; Rink et al., 1998; Wolf-Gladrow et al., 1999; Zeebe et

440   al., 2008), the boron isotopic composition and the B/Ca are faithful predictors of pH

441   and bicarbonate ion concentration, respectively. Our results provide strong evidence

442   that $[HCO_3^-]$ is recorded in the B/Ca ratio. A wide range of $[HCO_3^-]$ was necessary to

443   facilitate de-coupling the carbonate system from pH_T, however the high $[HCO_3^-]$ in

444   several of these treatments are unrealistic for natural seawater systems, particularly

445   paleo-systems which exhibit lower $[HCO_3^-]$ than present day oceans (calculated from

446   values given in Zeebe, 2012) and more environmentally-relevant values should be

447   used for future calibration experiments. The proxy should therefore be ground-truthed

448   using core top samples. The correlation of the B/Ca ratio to $[HCO_3^-]$ rather than to

449   $[CO_3^{2-}]$ might have some implications for existing paleo-carbonate chemistry

450   reconstructions based on this proxy such as the study by Foster (2008) and that of Yu

451   et al. (2014), since the same relationship probably holds for benthic foraminifers as

452   for planktonics.

453

454   A sound understanding of the effects of past carbon perturbations becomes

455   increasingly urgent in an age where anthropogenic activities are producing such rapid

456   changes in global climate (Bijma et al., 2013; Knoll and Fischer, 2011). The

457   usefulness of biogeochemical proxies to reconstruct paleoceanographic conditions is

458 well established for environmental parameters such as temperature and salinity

459 (Wefer et al., 1999) but uncertainties remain for proxies related to pH and the

460 carbonate system (Allen and Honisch, 2012; Hönisch et al., 2007; Katz et al., 2010;

461 Pagani et al., 2005). This study confirms the robustness of δ^{11}B as an independent pH

462 proxy and adds further weight to the growing body of evidence that B/Ca in

463 planktonic foraminiferal calcite may be used as an independent proxy for $[HCO_3^{2-}]$

464 (Yu et al. 2007), thereby allowing researchers to fully constrain the carbonate system.

465
466

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474

475 **References**
476
477 Allen, K. A. and Honisch, B.: The planktic foraminiferal B/Ca proxy for seawater

478 carbonate chemistry: A critical evaluation, Earth Planet Sci Lett, 345, 203-211, 2012.

479 Allen, K. A., Honisch, B., Eggins, S. M., and Rosenthal, Y.: Environmental controls

480 on B/Ca in calcite tests of the tropical planktic foraminifer species *Globigerinoides*

481 *ruber* and *Globigerinoides sacculifer*, Earth Planet Sci Lett, 351, 270-280, 2012.

482 Allen, K. A., Hönisch, B., Eggins, S. M., Yu, J., Spero, H. J., and Elderfield, H.:

483 Controls on boron incorporation in cultured tests of the planktic foraminifer *Orbulina*

484 *universa*, Earth Planet Sci Lett, 309, 291-301, 2011.

485 Bemis, B. E., Spero, H. J., Bijma, J., and Lea, D. W.: Reevaluation of the oxygen

486 isotopic compostion of planktonic foraminifera: Experimental results and revised

487 paleotemperature equations, Paleoceanography, 13, 150-160, 1998.

488 Bijma, J., Hemleben, C., Huber, B. T., Erlenkeuser, H., and Kroon, D.: Experimental

489 determination of the ontogenetic stable isotope variability in two morphotypes of

490 *Globigerinella siphonifera* (d'Orbigny), Mar. Micropaleontol., 35, 141-160, 1998.

491   Bijma, J., Pörtner, H. O., Yesson, C., and Rogers, A. D.: Climate change and the

492   oceans - what does the future hold?, Mar Pollut Bull, 74, 495-505, 2013.

493   Ciais, P., Sabine, C., Bala, G., Bopp, L., Brovkin, V., Canadell, J., Chhabra, A.,

494   DeFries, R., Galloway, J., Heimann, M., Jones, C., Le Quéré, C., Myneni, R. B., Piao,

495   S., and Thornton, P.: Carbon and Other Biogeochemical Cycles. In: Climate Change

496   2013: The Physical Science Basis. Contribution of Working Group I to the Fifth

497   Assessment Report of the Intergovernmental Panel on Climate Change, Stocker, T. F.,

498   Qin, D., Plattner, G.-K., Tignor, M., Allen, S. K., Boschung, J., Nauels, A., Xia, Y.,

499   Bex, V., and Midgley, P. M. (Eds.), Cambridge University Press, Cambridge, United

500   Kingdom and New York, NY, USA, 2013.

501   Dickson, A.: An exact definition of total alkalinity and a procedure for the estimation

502   of alkalinity and total inorganic carbon from titration data, Deep Sea Research Part A.

503   Oceanographic Research Papers, 28, 609-623, 1981.

504   Dickson, A. G.: The development of the alkalinity concept in marine chemistry, Mar.

505   Chem., 40, 49-63, 1992.

506   Dickson, A. G.: Thermodynamics of the dissociation of boric acid in synthetic

507   seawater from 273.15 to 318.15 K, Deep Sea Research Part A. Oceanographic

508   Research Papers, 37, 755-766, 1990.

509   Dickson, A. G., Sabine, C. L., Christian, J. R., and (Eds.): Guide to best practices for

510   ocean CO_2 measurements, 191 pp., 2007.

511   DOE: Handbook of methods for the analysis of the various parameters of the cabon

512   dioxide system in seawater; version 2, ORNL/CDIAC-74, 1994.

513   Foster, G. L.: Seawater pH, pCO_2 and $[CO_3^{-2}]$ variations in the Caribbean Sea over

514   the last 130 kyr: A boron isotope and B/Ca study of planktic foraminifera, Earth

515   Planet Sci Lett, 271, 254-266, 2008.

516   Foster, G. L., Lear, C. H., and Rae, J. W. B.: The evolution of pCO_2, ice volume and

517   climate during the middle Miocene, Earth Planet Sci Lett, 341, 243-254, 2012.

518   Foster, G. L., Ni, Y. Y., Haley, B., and Elliott, T.: Accurate and precise isotopic

519   measurement of sub-nanogram sized samples of foraminiferal hosted boron by total

520   evaporation NTIMS, Chemical Geology, 230, 161-174, 2006.

521   Gattuso, J.-P. and Hansson, L.: Ocean acidification: background and history. In:

522   Ocean Acidification, Gattuso, J.-P. and Hansson, L. (Eds.), Oxford University Press,

523   New York, NY, USA, 2011.

524 Hemming, N. G., Guilderson, T. P., and Fairbanks, R. G.: Seasonal variations in the
525 boron isotopic composition of coral: A productivity signal?, Global Biogeochem Cy,
526 12, 581-586, 1998.

527 Hemming, N. G. and Hanson, G. N.: Boron isotopic composition and concentration in
528 modern marine carbonates, Geochim. Cosmochim. Acta, 56, 537-543, 1992.

529 Henehan, M. J., Foster, G. L., Rae, J. W., Prentice, K. C., Erez, J., Bostock, H. C.,
530 Marshall, B. J., and Wilson, P. A.: Evaluating the utility of B/Ca ratios in planktic
531 foraminifera as a proxy for the carbonate system: A case study of *Globigerinoides*
532 *ruber*, Geochemistry, Geophysics, Geosystems, 16, 1052-1069, 2015.

533 Hönisch, B., Allen, K., Hyams, O., Penman, D., Raitzsch, M., Ruprecht, J., Thomas,
534 E., and Zachos, J.: Ocean acidification during the Cenozoic, Appl. Geochem., 26,
535 S288-S288, 2011.

536 Hönisch, B., Bickert, T., and Hemming, N. G.: Modern and Pleistocene boron isotope
537 composition of the benthic foraminifer *Cibicidoides wuellerstorfi*, Earth Planet Sci
538 Lett, 272, 309-318, 2008.

539 Hönisch, B., Bijma, J., Russell, A. D., Spero, H. J., Palmer, M. R., Zeebe, R. E., and
540 Eisenhauer, A.: The influence of symbiont photosynthesis on the boron isotopic
541 composition of foraminifera shells, Mar. Micropaleontol., 49, 87-96, 2003.

542 Hönisch, B. and Hemming, N. G.: Surface ocean pH response to variations in pCO_2
543 through two full glacial cycles, Earth Planet Sci Lett, 236, 305-314, 2005.

544 Hönisch, B., Hemming, N. G., Archer, D., Siddall, M., and McManus, J. F.:
545 Atmospheric carbon dioxide concentration across the mid-Pleistocene transition,
546 Science, 324, 1551-1554, 2009.

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

550 Hönisch, B., Hemming, N. G., and Loose, B.: Comment on "A critical evaluation of
551 the boron isotope-pH proxy: The accuracy of ancient ocean pH estimates" by M.
552 Pagani, D. Lemarchand, A. Spivack and J. Gaillardet, Geochim. Cosmochim. Acta,
553 71, 1636-1641, 2007.

554 Hönisch, B., Ridgwell, A., Schmidt, D. N., Thomas, E., Gibbs, S. J., Sluijs, A., Zeebe,
555 R. E., Kump, L., Martindale, R. C., Greene, S. E., Kiessling, W., Ries, J. B., Zachos,
556 J. C., Royer, D. L., Barker, S., Marchitto Jr., T. M., Moyer, R., Pelejero, C., Ziveri, P.,

557 Foster, G. L., and Williams, B.: The geological record of ocean acidification, Science,

558 335, 1302-1302, 2012.

559 Hoppe, C. J. M., Langer, G., Rokitta, S. D., Wolf-Gladrow, D. A., and Rost, B.:

560 Implications of observed inconsistencies in carbonate chemistry measurements for

561 ocean acidification studies, Biogeosciences, 9, 2401-2405, 2012.

562 Kaczmarek, K., Horn, I., Nehrke, G., and Bijma, J.: Simultaneous determination of

563 $\delta^{11}B$ and B/Ca ratio in marine biogenic carbonates at nanogram level, Chemical

564 Geology, 392, 32-42, 2015a.

565 Kaczmarek, K., Langer, G., Nehrke, G., Horn, I., Misra, S., Janse, M., and Bijma, J.:

566 Boron incorporation in the foraminifer *Amphistegina lessonii* under a decoupled

567 carbonate chemistry, Biogeosciences, 12, 1753-1763, 2015b.

568 Katz, M. E., Cramer, B. S., Franzese, A., Hönisch, B., Miller, K. G., Rosenthal, Y.,

569 and Wright, J. D.: Traditional and emerging geochemical proxies in foraminifera, J.

570 Foraminifer. Res., 40, 165-192, 2010.

571 Klochko, K., Cody, G. D., Tossell, J. A., Dera, P., and Kaufman, A. J.: Re-evaluating

572 boron speciation in biogenic calcite and aragonite using 11B MAS NMR, Geochim.

573 Cosmochim. Acta, 73, 1890-1900, 2009.

574 Klochko, K., Kaufman, A. J., Yao, W., Byrne, R. H., and Tossell, J. A.: Experimental

575 measurement of boron isotope fractionation in seawater, Earth Planet Sci Lett, 248,

576 276-285, 2006.

577 Knoll, A. H. and Fischer, W. W.: Skeletons and ocean chemistry: the long view. In:

578 Ocean Acidification, Gattuso, J.-P. and Hansson, L. (Eds.), Oxford University Press,

579 New York, NY, USA, 2011.

580 Köhler-Rink, S. and Kuhl, M.: Microsensor studies of photosynthesis and respiration

581 in the larger symbiont bearing foraminifera *Amphistegina lobifera*, and *Amphisorus*

582 *hemprichii*, Ophelia, 55, 111-122, 2001.

583 Köhler-Rink, S. and Kühl, M.: Microsensor studies of photosynthesis and respiration

584 in larger symbiotic foraminifera. I The physico-chemical microenviron-ment of

585 *Marginopora vertebralis*, *Amphistegina lobifera* and *Amphisorus hemprichii*, Mar.

586 Biol., 137, 473-486, 2000.

587 Lavigne, H. and Gattuso, J.-P.: Seacarb 1.2.3., an R package to calculate parameters

588 of the seawater carbonate system, , 2013. online available at: http://cran.at.r-

589 project.org/web/packages/seacarb/index.html, 2013.

590   Longerich, H. P., Jackson, S. E., and Günther, D.: Inter-laboratory note. Laser

591   ablation inductively coupled plasma mass spectrometric transient signal data

592   acquisition and analyte concentration calculation, J Anal Atom Spectrom, 11, 899-

593   904, 1996.

594   Martínez-Botí, M., Foster, G., Chalk, T., Rohling, E., Sexton, P., Lunt, D., Pancost,

595   R., Badger, M., and Schmidt, D.: Plio-Pleistocene climate sensitivity evaluated using

596   high-resolution CO_2 records, Nature, 518, 49-54, 2015.

597   Mehrbach, C., Culberso.Ch, Hawley, J. E., and Pytkowic.Rm: Measurement of

598   apparent dissociation-constants of carbonic-acid in seawater at atmospheric-pressure,

599   Limnol. Oceanogr., 18, 897-907, 1973.

600   Pagani, M., Lemarchand, D., Spivack, A., and Gaillardet, J.: A critical evaluation of

601   the boron isotope-pH proxy: The accuracy of ancient ocean pH estimates, Geochim.

602   Cosmochim. Acta, 69, 953-961, 2005.

603   Palmer, M. R., Pearson, P. N., and Cobb, S. J.: Reconstructing past ocean pH-depth

604   profiles, Science, 282, 1468-1471, 1998.

605   Palmer, M. R., Spivack, A. J., and Edmond, J. M.: Temperature and pH controls over

606   the fractionation during adsorption of boron on marine clay, Geochim. Cosmochim.

607   Acta, 51, 2319-2323, 1987.

608   Pearson, P. N. and Palmer, M. R.: Atmospheric carbon dioxide concentrations over

609   the past 60 million years, Nature, 406, 695-699, 2000.

610   Pearson, P. N. and Palmer, M. R.: Middle Eocene seawater pH and atmospheric

611   carbon dioxide concentrations, Science, 284, 1824-1826, 1999.

612   R Core Team: R: A language and environment for statistical computing., R

613   Foundation for Statistical Computing, Vienna, Austria, 2008.

614   Rae, J. W. B., Foster, G. L., Schmidt, D. N., and Elliott, T.: Boron isotopes and B/Ca

615   in benthic foraminifera: Proxies for the deep ocean carbonate system, Earth Planet Sci

616   Lett, 302, 403-413, 2011.

617   Rhein, M., Rintoul, S. R., Aoki, S., Campos, E., Chambers, D., Feely, R. A., Gulev,

618   S., Johnson, G. C., Josey, S. A., Kostianoy, A., Mauritzen, C., Roemmich, D., Talley,

619   L. D., and Wang, F.: Observations: Ocean. In: Climate Change 2013: The Physical

620   Science Basis. Contribution of Working Group I to the Fifth Assessment Report of

621   the Intergovernmental Panel on Climate Change, Stocker, T. F., Qin, D., Plattner, G.-

622   K., Tignor, M., Allen, S. K., Boschung, J., Nauels, A., Xia, Y., Bex, V., and Midgley,

623 P. M. (Eds.), Cambridge University Press, Cambridge, United Kingdom and New

624 York, NY, USA, 2013.

625 Rink, S., Kühl, M., Bijma, J., and Spero, H. J.: Microsensor studies of photosynthesis

626 and respiration in the symbiotic foraminifer *Orbulina universa*, Mar. Biol., 131, 583-

627 595, 1998.

628 Sanyal, A., Bijma, J., Spero, H. J., and Lea, D. W.: Empirical relationship between pH

629 and the boron isotopic composition of *Globigerinoides sacculifer*: Implications for the

630 boron isotope paleo-pH proxy, Paleoceanography, 16, 515-519, 2001.

631 Sanyal, A., Hemming, N. G., Broecker, W. S., and Hanson, G. N.: Changes in pH in

632 the eastern equatorial pacific across stage 5-6 boundary based on boron isotopes in

633 foraminifera, Global Biogeochem Cy, 11, 125-133, 1997.

634 Sanyal, A., Hemming, N. G., Broecker, W. S., Lea, D. W., Spero, H. J., and Hanson,

635 G. N.: Oceanic pH control on the boron isotopic composition of foraminifera:

636 Evidence from culture experiments, Paleoceanography, 11, 513-517, 1996.

637 Sanyal, A., Hemming, N. G., Hanson, G. N., and Broecker, W. S.: Evidence for a

638 higher pH in the glacial ocean from boron isotopes in foraminifera, Nature, 373, 234-

639 236, 1995.

640 Sanyal, A., Nugent, M., Reeder, R. J., and Bijma, J.: Seawater pH control on the

641 boron isotopic composition of calcite: Evidence from inorganic calcite precipitation

642 experiments, Geochim. Cosmochim. Acta, 64, 1551-1555, 2000.

643 Siegenthaler, U. and Sarmiento, J. L.: Atmospheric carbon dioxide and the ocean,

644 Nature, 365, 119-125, 1993.

645 Spero, H. J. and Lea, D. W.: Intraspecific stable-isotope variability in the planktic

646 foraminifera *Globigerinoides-sacculifer* - Results from laboratory experiments, Mar.

647 Micropaleontol., 22, 221-234, 1993.

648 Spivack, A. J., You, C.-F., and Smith, H. J.: Foraminiferal boron isotope ratios as a

649 proxy for surface ocean pH over the past 21 Myr, Nature, 363, 149-151, 1993.

650 Van Andel, T. H.: Mesozoic/Cenozoic calcite compensation depth and the global

651 distribution of calcareous sediments, Earth Planet Sci Lett, 26, 187-194, 1975.

652 Vengosh, A., Kolodny, Y., Starinski, A., Chivas, A. R., and McCulloch, M. T.:

653 Coprecipitation and isotopic fractionation of boron in modern biogenic carbonates,

654 Geochim. Cosmochim. Acta, 55, 2901-2910, 1991.

655   Wefer, G., Berger, W. H., Bijma, J., and Fischer, G.: Clues to ocean history: a brief

656   overview of proxies. In: Use of proxies in paleoceanography: Examples from the

657   South Atlantic, Fischer, G. and Wefer, G. (Eds.), Springer-Verlag, Berlin, Heidelberg,

658   1999.

659   Wolf-Gladrow, D. A., Bijma, J., and Zeebe, R. E.: Model simulation of the carbonate

660   chemistry in the microenvironment of symbiont bearing foraminifera, Mar. Chem.,

661   64, 181-198, 1999.

662   Wolf-Gladrow, D. A., Zeebe, R. E., Klaas, C., Körtzinger, A., and Dickson, A. G.:

663   Total alkalinity: The explicit conservative expression and its application to

664   biogeochemical processes, Mar. Chem., 106, 287-300, 2007.

665   Yu, J., Anderson, R., and Rohling, E.: Deep Ocean Carbonate Chemistry and Glacial-

666   Interglacial Atmospheric CO_2 Change, Oceanography, 27, 16-25, 2014.

667   Zeebe, R. E.: History of Seawater Carbonate Chemistry, Atmospheric CO_2, and

668   Ocean Acidification, Annu Rev Earth Pl Sc, 40, 141-165, 2012.

669   Zeebe, R. E.: Vital effects in foraminifera do not compromise the use of $\delta^{11}B$ as a

670   paleo-pH indicator: Evidence from modeling, Paleoceanography, 18, 2003.

671   Zeebe, R. E., Bijma, J., Hönisch, B., Sanyal, A., Spero, H. J., and Wolf-Gladrow, D.

672   A.: Vital effects and beyond: a modelling perspective on developing

673   palaeoceanographical proxy relationships in foraminifera. In: Biogeochemical

674   Controls on Palaeoceanographic Environmental Proxies, Austin, W. E. N. and James,

675   R. H. (Eds.), Geological Society of London, Special Publications, London, 2008.

676   Zeebe, R. E., Sanyal, A., Ortiz, J. D., and Wolf-Gladrow, D. A.: A theoretical study of

677   the kinetics of the boric acid - borate equilibrium in seawater, Mar. Chem., 73, 113-

678   124, 2001.

679   Zeebe, R. E. and Wolf-Gladrow, D. A.: CO_2 in Seawater: Equilibrium, Kinetics,

680   Isotopes, Elsevier, Amsterdam, The Netherlands, 2001.

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694 **Tables and table legends**
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697 Table 1: Average properties of the manipulated seawater culture medium from 4
698 samples (2 from the start of the incubation and two from the end of the incubation).
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pH_T	C_T (μmol kg^{-1})	A_T (μmol kg^{-1})	pCO_2 (μatm)	CO_3^{2-} (μmol kg^{-1})	HCO_3^- (μmol kg^{-1})	T (°C)	S	δ^{11}B (‰)
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 ± 0.02	2235.9	2566.8 ± 11	431.8	238.7	1981	23 ± 0.7	38 ± 0.6	5.35 ± 0.53
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

700
701 Table 2: Instrumental operating conditions for the MC-ICP-MS and LA.
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703

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

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713   Table 3: Mean values and 2SE of B/Ca and δ^{11}B values for the different experimental

714   treatments.

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pH$_T$	CO$_3$$^{2-}$ (μmol kg^{-1})	δ^{11}B (‰)	B/Ca	N samples
8.05 ± 0.05	285.6	19.1 (1.27)	1.49 (0.06)	13
8.05 ± 0.02	238.7	17.8 (1.8)	1.27 (0.08)	9
8.05 ± 0.03	533.9	20 (2.24)	0.77 0.03)	12
7.9 ± 0.02	296.6	16.8 (1.29)	0.92 (0.05)	18
7.7 ± 0.03	257.8	14.9 (1.44)	0.69 (0.04)	18

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752   **Figures and legends**

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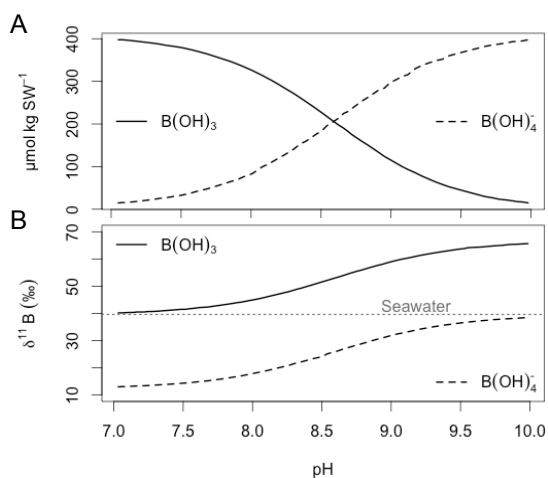
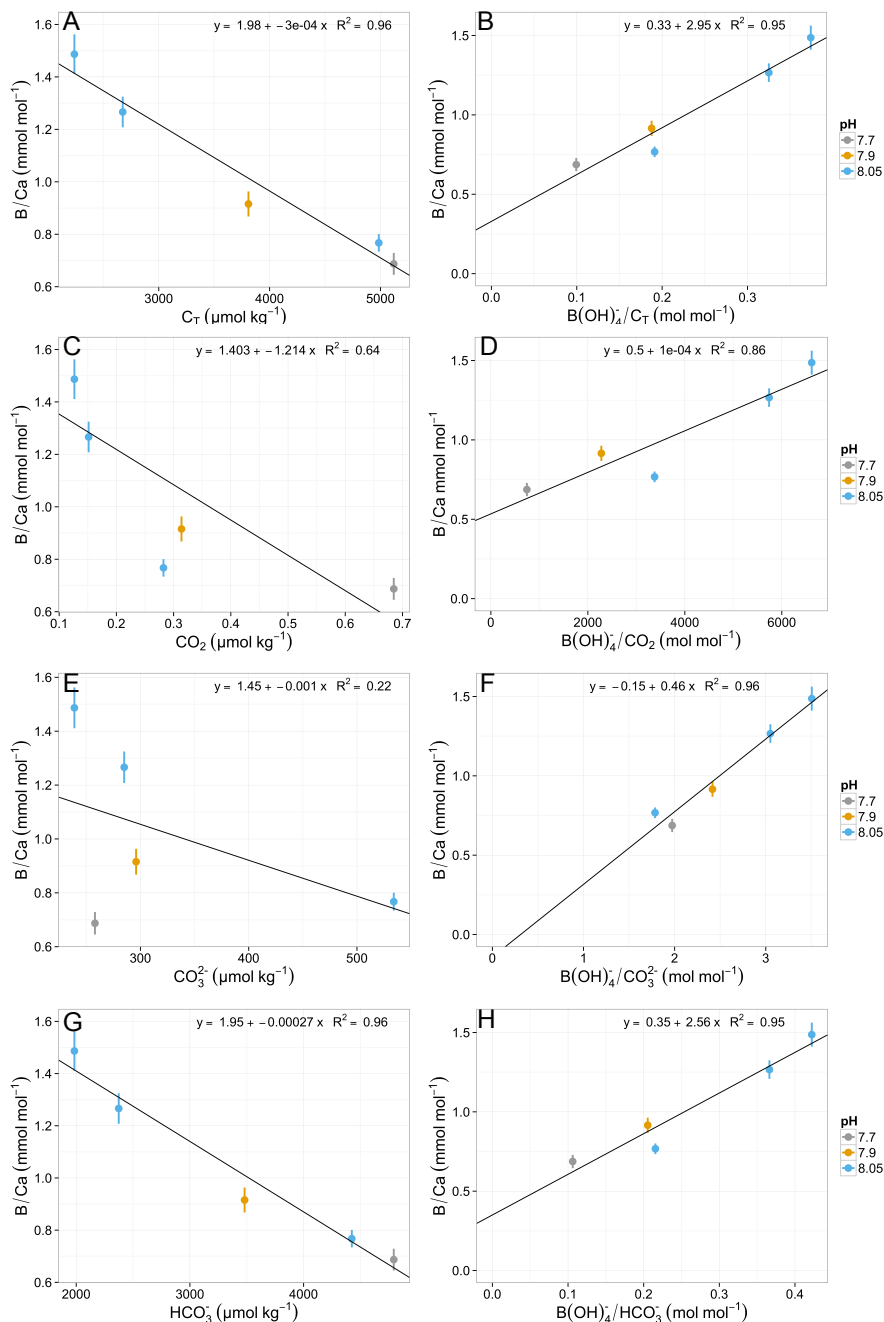

758   Fig 1: (A) Bjerrum plot showing the effect of pH on concentration of dissolved boron

759   species at T = 25°C, S = 35 and [B] $_{total}$ 416 μmol kg^{-1}. (B) Effect of pH on boron

760   isotopic composition of B(OH)$_4^-$ and B(OH)$_3$ with thermodynamic fractionation factor

761   (α_{3-4}) = 1.030 (Hemming & Hanson 1992).

763   Fig. 2: B/Ca ratios plotted against (A) [C_T], (B) [$B(OH)_4^-$]/[C_T] ratio, (C) [CO_2], (D)
764   [$B(OH)_4^-$]/[CO_2], (E) [CO_3^{2-}], (F) [$B(OH)_4^-$]/[CO_3^{2-}], (G) [HCO_3^-], (H) [$B(OH)_4^-$]/[
765   HCO_3^-], error bars represent standard error.

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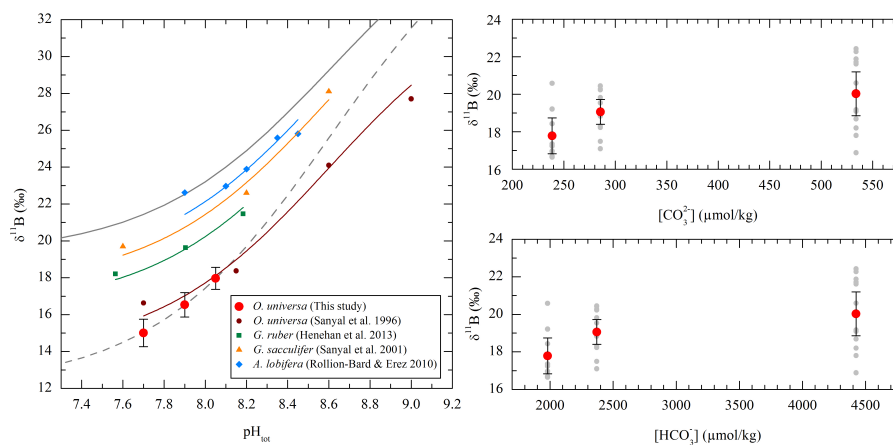
Fig 3: (A) Median and converted $\delta^{11}B$ of cultured *O. universa* calcite (black circles)
error bars represent ±2 standard errors, solid grey line shows empirical values for
seawater $\delta^{11}B_{borate}$ with a fractionation factor of $^{11\text{-}10}K_B = 1.020$ (Hönisch et al.,
2007) at T=23°C 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., 2009) at T=23°C and
S=38. (B) Median $\delta^{11}B$ of $B(OH)_4^-$ in cultured *O. universa* calcite grown at constant
pH of 8.05 but varying $[CO_3^{2-}]/[HCO_3^-]$. Error bars represent ±2 standard
errors across all single laser ablation analyses per treatment.

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