1 **The pH dependency of the boron isotopic composition of diatom opal**

2 **(*Thalassiosira weissflogii*)**

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15 **Abstract**
16 The high latitude oceans are key areas of carbon and heat exchange between the atmosphere and the
17 ocean. As such, they are a focus of both modern oceanographic and palaeoclimate research. However,
18 most palaeoclimate proxies that could provide a long-term perspective are based on calcareous
19 organisms, such as foraminifera, that are scarce or entirely absent in deep-sea sediments south of
20 50°S in the Southern Ocean and north of 40°N in the North Pacific. As a result, proxies need to be
21 developed for the opal-based organisms (*e.g.* diatoms) found at these high latitudes, which dominate
22 the biogenic sediments recovered from these regions. Here we present a method for the analysis of
23 the boron (B) content and isotopic composition ($\delta^{11}B$) of diatom opal. We apply it for the first time to
24 evaluate the relationship between seawater pH, $\delta^{11}B$ and B concentration ([B]) in the frustules of the
25 diatom *Thalassiosira weissflogii,* cultured across a range of carbon dioxide partial pressure (pCO$_2$) and
26 pH values. In agreement with existing data, we find that the [B] of the cultured diatom frustules
27 increases with increasing pH (Mejia et al., 2013). $\delta^{11}B$ shows a relatively well-defined negative trend
28 with increasing pH, completely distinct from any other biomineral previously measured. This
29 relationship not only has implications for the magnitude of the isotopic fractionation that occurs
30 during boron incorporation into opal, but also allows us to explore the potential of the boron-based
31 proxies for palaeo-pH and palaeo-CO$_2$ reconstruction in high latitude marine sediments that have, up
32 until now, eluded study due to the lack of suitable carbonate material.

33
34 **1. Introduction**

35 The high latitude regions, such as the Southern Ocean and the subarctic North Pacific Ocean, exert

36 key controls on atmospheric carbon dioxide (CO$_2$) content. Both areas are where upwelling of deep

37 carbon- and nutrient-rich water occurs, which promotes outgassing of previously stored carbon to the

38 atmosphere and nutrient fertilisation of primary productivity, in turn drawing down CO$_2$. The balance

39 of processes involved in determining whether these oceanic regions are a source or sink of CO$_2$ are

40 poorly understood, to the extent that the oceanic controls on glacial-interglacial pH and pCO$_2$ changes

41 remain a subject of vigorous debate (*e.g.* Martin, 1990; Sigman and Boyle, 2000). Recently, several

42 studies have shown how the boron isotope pH proxy applied to calcitic foraminifera successfully tracks

43 surface water CO_2 content, thus documenting changes in air-sea CO_2 flux along the margins of these

44 regions (*e.g.* Martínez-Botí et al., 2015; Gray et al. 2018). However, the lack of preserved marine

45 carbonates in areas that are thought to be key in terms of glacial-interglacial CO_2 change (*e.g.* the

46 polar Antarctic zone; Sigman et al., 2010) represents a currently insurmountable problem, preventing

47 the determination of air-sea CO_2 flux using boron-based proxies in regions that are likely to play the

48 most important role in glacial-interglacial CO_2 change. There is therefore a clear need for the boron

49 isotope palaeo-pH proxy to be developed in biogenic silica (diatom frustules, radiolarian shells), which

50 is preserved in high-latitude settings, to better understand these key regions and their role in natural

51 climate change.

52

53 The boron isotopic system has been used extensively in marine carbonates for the reconstruction of

54 past ocean pH and past atmospheric $p$$CO_2$ (*e.g.* Hemming and Hanson, 1992; Pearson and Palmer,

55 2000; Hönisch and Hemming, 2005; Foster, 2008; Henehan et al., 2013; Chalk et al. 2017; Sosdian et

56 al. 2018). Comprehensive calibration work has been completed for numerous species of foraminifera

57 that are currently used in palaeoceanographic reconstruction (*e.g.* Henehan et al. 2016; Rae et al.

58 2011). From this it has been shown that while $\delta^{11}B$ compositions are fairly similar among carbonates,

59 species-specific differences exist in the relationship between the $\delta^{11}B$ of dissolved borate and that of

60 foraminifera. Once this relationship is known, this $\delta^{11}B$-pH calibration can be applied to fossils found

61 in deep-sea sediment cores, reliably reconstructing past ocean pH and $p$$CO_2$ (*e.g.* Hönisch and

62 Hemming, 2005; Foster, 2008, Hönisch et al., 2009; Chalk et al., 2017). However, thus far the boron

63 isotopic composition (expressed as $\delta^{11}B$) and B concentration ([B]) of the siliceous fraction of deep-

64 sea sediments remains poorly studied.

65

66 Early exploratory work by Ishikawa and Nakamura (1993) showed that biogenic silica and diatom ooze

67 collected from modern deep-sea sediments in the North and Equatorial Pacific had relatively high

68 boron contents (70-80 ppm), but a very light isotope ratio. For example, a diatom ooze was shown to

69 have a $\delta^{11}B$ of -1.1 ‰ whilst radiolarian shells had a $\delta^{11}B$ of +4.5 ‰. While some of this light $\delta^{11}B$ may

70 have partly arisen due to clay contamination (reducing the diatom ooze sample by up to 3 ‰; Ishikawa

71 and Nakamura, 1993) it also likely reflects an opal:seawater isotopic fractionation arising from the

72 substitution of borate for silicate in tetrahedral sites in the opal (Ishikawa and Nakamura, 1993). A

73 similarly light $\delta^{11}B$ was also observed in marine cherts from deep sea sediments by Kolodny and

74 Chaussidon (2004; -9.3 to +8 ‰), but these are likely diagenetic and therefore unlikely to be primary

seawater precipitates. A recent culture study of the diatoms *Thalassiosira weissflogii* and *T. pseudonana* showed that the boron content of cultured opal was significantly lower than suggested by the bulk sampling of Ishikawa and Nakamura (1993) at around 5-10 ppm, increasing as pH increased from 7.6 to 8.7 (Mejia et al., 2013). This suggests seawater tetrahydroxyborate anion (borate; $B(OH)_4^-$) is predominantly incorporated into the diatom frustule rather than boric acid ($B(OH)_3$) and implies there is potential for the boron content of diatom opal to trace pH in the past (Mejia et al., 2013).

Here, the relationship between $\delta^{11}B$ of the frustules of the diatom *T. weissflogii* and seawater pH is investigated for the first time using a batch culturing technique and different air-CO_2 mixtures to explore a range of pH (8.54 ± 0.57 to 7.48 ± 0.06). The aim of this study was also to develop a methodology for measuring the boron isotopic composition of biogenic silica by MC-ICP-MS and apply this method to explore the response of the boron-based proxies ([B] and $\delta^{11}B$) in diatom frustules to changing pH. Ultimately, we show how boron isotopes measured in diatom frustules may provide further insight into boron uptake and physiological activity within diatoms and test the potential of $\delta^{11}B$ and boron content in diatoms as proxies for the ocean carbonate system.

2. Methods

2.1 Experimental Set up

The centric diatom *T. weissflogii* (Grunow in van Heurck, PCC 541, CCAP 1085/1; Hasle and Fryxell, 1977) was grown in triplicate in enriched sterile and filtered seawater (K/1; 0.2 μm; seawater sourced from Labrador Sea; Keller et al., 1987) in 3 L glass Erlenmeyer flasks for a maximum of one week for each experiment. Initial nutrient concentrations within the seawater before enrichment were assessed on a SEAL Analytical QuAAtro analyser with a UV/vis spectrometer and ranged from 23.3 to 27.5 µM for nitrate(+nitrite), 4.3 to 5.4 µM for silicic acid and 1.4 to 1.6 µM for phosphate. The culture experiments were bubbled with air-CO_2 mixtures in different concentrations (sourced from BOC; ; www.boconline.co.uk) to provide a pH range at constant bubble rates, and every flask was agitated by hand twice daily to limit algal settling and aggregation. The monocultures were grown in nutrient replete conditions at constant temperature (20°C) and on a 12h:12 h light:dark cycle (with 192 μE m^{-2} s^{-1}, or 8.3 E m^{-2} d^{-1} during the photoperiod). The diatoms were acclimated to each pCO$_2$ treatment for at least 10 generations before inoculating the culture experiment flasks. All culture handling was completed within a laminar flow hood to ensure sterility. The flow hood surfaces were cleaned with 90% ethanol before and after handling, as well as the outer surface of all autoclaved labware entering the laminar flow hood such as bottles and pipettes.

109   The cultured diatom samples were collected by centrifugation at 96 h, during the exponential growth

110   phase. Each flask was simultaneously disconnected from the gas supply with the culture immediately

111   centrifuged at 3700 rpm for 30 minutes into a pellet, rinsed with MilliQ, and frozen at -20°C in sterile

112   plastic 50 mL centrifuge tubes. Around 10 mg of diatom biomass was harvested in each experiment.

113

114   **2.2. Growth rate and cell size**

115   A 5 mL sub-sample was taken from each culture flask through sterilised Nalgene tubing into sterile

116   syringes and sealed in sterile 15 mL centrifuge tubes. Triplicate cell counts using a Coulter

117   Multizier^{TM3} (Beckman Coulter) were performed daily on each experimental flask. Growth rates were

118   calculated using equation 1:

119    $$\mu = (lnN_t - lnN_i)/(t - t_i) \qquad\qquad (1)$$

120   where N_i is the initial cell density at the start of the experiment (t_i) and N_t is the cell density at time t.

121   Triplicate estimates of cell size were also determined using the Coulter Multizier^{TM3}, to determine

122   the mean cell size over time in each flask. Figure 1 shows that although there is no statistically

123   significant relationship between pH and diatom growth rate, cell size does show a small, but

124   statistically significant, positive slope.

125   **2.3 pH, DIC and δ^{11}B of the culture media**

126   A pH meter (Orion 410A) calibrated using standard National Bureau of Standards (NBS) buffers prior

127   to sample extraction was used to monitor the evolution of pH through the experiment on a daily

128   basis. For fully quantitative constraints on the carbonate system of the culture media, dissolved

129   inorganic carbon (DIC) was measured in triplicate, every other day, for each pH treatment (*i.e.* once

130   per experiment flask). The 100 mL sample bottles were filled to overflowing and immediately closed

131   with ground glass stoppers, then uncapped to be poisoned with 20 µL saturated mercuric chloride

132   solution (HgCl$_2$) to prevent any further biologically-induced changes in DIC, before being sealed with

133   a 1 mL air headspace and Apiezon L grease, and stored in complete darkness until analysis (Dickson

134   et al., 2007). Analysis of DIC was performed by acidification with excess 10% phosphoric acid and CO$_2$

135   transfer in a nitrogen gas stream to an infrared detector using a DIC Analyzer AS-C3 (Apollo SciTech,

136   DE, USA) at the University of Southampton. The DIC results were calibrated using measurements of

137   batch 151 certified reference material obtained from A. G. Dickson (Scripps Institution of

138   Oceanography, CA, USA). The accuracy of the DIC analysis was ca. 3 µmol kg^{-1}. Carbonate system

139   parameters, including seawater pCO$_2$, were calculated using measured pH$_{NBS}$ and DIC values,

140 temperature, salinity and nutrients with the CO_2SYS v1.1 program (van Heuven et al., 2011; using

141 constants from Dickson, 1990; Lueker et al., 2000; Lee et al., 2010), which was also used to convert

142 pH meter readings from the NBS to the Total scale (used throughout).

143 All flasks were initially filled with media from the same large batch and all culture treatments

144 therefore started with the same initial pH. The pH for all treatments was then altered by bubbling

145 through the different air-CO_2 mixtures, ranging from low pH (target = 1600 ppm, high pCO$_2$) to high

146 pH (target = 200 ppm, low pCO$_2$). Almost all treatments held relatively constant DIC and pH until the

147 final 24 hours of the experiment, when marked changes in DIC and pH in all culture treatments were

148 observed (Figure 2), which in most cases was likely due to the growth of diatoms and an associated

149 net removal of DIC, despite the constant addition of pCO$_2$. In order to account for these non-steady

150 state conditions of the carbonate system, the mean pH and pCO$_2$ of each treatment were calculated

151 based on the number of cells grown per 24 hours along with the pH/pCO$_2$ measured in that 24 hours,

152 thus adjusting for the observed exponential growth rate of *T. weissflogii* (Table 1).

153

154 The boron concentration of the culture media was not determined but is assumed to be the same as

155 Labrador seawater (~4.5 ppm; Lee et al., 2010). The boron isotopic composition of the culture media

156 was determined using standard approaches (Foster et al., 2010) to be 38.8 ± 0.19 ‰ (2 s.d.).

157 **2.4 Preparing cultured diatoms for δ^{11}B and B/Si analysis**

158 In order to examine reproducibility and accuracy of our boron measurements, an in-house diatom

159 reference material was used to develop a method for measuring boron isotopes and boron

160 concentration in biogenic silica. A British Antarctic Survey core catcher sample (TC460) from core

161 TC460 in the Southern Ocean (-60.81534° N, -50.9851° E, water depth 2594 m) was used for this

162 purpose (supplied by C.-D. Hildebrand [British Antarctic Survey]). Although the diatom assemblage

163 was not characterised in the core catcher, the nearest sediment sample in the core is dominated by

164 *Hyalochaete Chaetoceros* resting spores, representing circa 70% of the total diatom content, with sea

165 ice and cool open water species making up the bulk of the remaining 30% (*e.g. Actinocyclus*

166 *actinochilus, Fragilariopsis curta, F. cylindrus, F. obliquecostata, Odontella weissflogii, Thalassiosira*

167 *antarctica*). A pure diatom sample of mixed species was separated from this bulk sediment and

168 cleaned of clay contamination at the University of Nottingham following an established diatom

169 separation technique (Swann et al., 2013). Briefly, the bulk sample underwent organic removal and

170 carbonate dissolution (using 30% H_2O_2 and 5% HCl), heavy liquid separation in several steps at

171 different specific gravities using sodium polytungstate (SPT) and visual monitoring throughout the

172 process to ensure the sample was free from non-diatom material, such as clay particulates. After the

173 final SPT separation, samples were rinsed thoroughly with MilliQ and sieved at 10 µm to remove all
174 SPT traces.

175

176 The culture samples and the diatom fraction from TC460 were first acidified (H_2SO_4) and organics were
177 oxidised using potassium permanganate and oxalic acid (following Horn et al., 2011 and Mejía et al.,
178 2013). The samples were rinsed thoroughly using MilliQ water via centrifugation and transferred to
179 acid-cleaned Teflon beakers. A secondary oxidation was completed under heat using perchloric acid.
180 Finally, the organic-free samples were rinsed thoroughly with MilliQ via filtration.

181

182 In the boron-free HEPA filtered clean laboratory at the University of Southampton, each sample was
183 dissolved completely in a gravimetrically known amount of NaOH (0.5 M from 10 M concentrated
184 stock supplied by Fluka) at 140°C for 6 to 12 h and briefly centrifuged prior to boron separation to
185 ensure no insoluble particles were loaded onto the boron column. Anion exchange columns containing
186 Amberlite IRA 743 resin were then used to separate the matrix from the boron fraction of each sample
187 following Foster (2008). Briefly, the dissolved opal was loaded directly onto the column without
188 buffering and the matrix removed with 9 x 200 µL washes of MilliQ. This was collected for subsequent
189 analysis and the pure boron fraction was then eluted and collected in 550 µL of 0.5 M HNO_3 acid. The
190 level of potential contamination was frequently monitored using total procedural blanks (TPB)
191 measured in every batch of columns. The TPB comprised an equivalent volume of sodium hydroxide
192 (NaOH, 0.5 M) as used in the samples of each batch (ca. 0.2 - 4 mL). This was analysed following the
193 sample analysis protocols detailed below, typically the TPBs for this work contained less than 40 pg of
194 boron. This equates to a typical blank contribution of ca. 0.015%, which results in a negligible
195 correction and is therefore ignored here.

196

197 Prior to isotope analysis, all boron fractions were collected in pre-weighed acid cleaned Teflon beakers
198 and their mass was recorded using a Precisa balance. A 10 µL aliquot was taken and diluted with 490
199 µL 0.5 M HNO_3 in acid cleaned plastic centrifuge tubes (2 mL). This was then analysed using a Thermo
200 Fisher Scientific Element 2XR ICP-MS at the University of Southampton, with boron concentration
201 determined using standard approaches and a gravimetric standard containing boron, silicon, sodium
202 and aluminium. In order to determine the B/Si ratio and hence the B concentration of the opal, the Si
203 concentration must also be quantitatively measured. This is achieved here by using a known
204 concentration and mass of NaOH to dissolve each sample, by measuring the Si/Na ratio the Si
205 concentration of each opal sample can be determined. From this, assuming a chemical formula of
206 $SiO_2.H_2O$ and a H_2O content of 8% (Hendry and Anderson, 2013), the B content of the opal in ppm can

207   be estimated. As detailed above, during the purification procedure, sample matrix was washed off the

208   column using MilliQ and collected in pre-weighed acid cleaned Teflon beakers. These samples were

209   then diluted with 3 % HNO$_3$ enriched with Be, In and Re for the internal standardisation and measured

210   on the Thermo Scientific X-series ICP-MS. The standards run on the X-Series consisted of varied

211   concentrations of the gravimetric standard also used on the Element, containing B, Si, Na and Al.

212

213   The boron isotopic composition of the biogenic silica samples was determined on a Thermo Scientific

214   Neptune MC-ICP-MS, also situated in a boron-free HEPA filtered laboratory at the University of

215   Southampton, following Foster (2008). Instrument induced fractionation of the ^{11}B/^{10}B ratio was

216   corrected using a sample-standard bracketing routine with NIST SRM 951, following Foster (2008).

217   This allows a direct determination of δ^{11}B without recourse to an absolute value for NIST SRM 951

218   (Foster, 2008) using the following equation, where ^{11}B/^{10}B$_{standard}$ is the mean ^{11}B/^{10}B ratio of the

219   standards bracketing the sample of interest.

220

221   $$\delta^{11}\text{B} = \left[\left(\frac{^{11}\text{B}/^{10}\text{B}_{\text{sample}}}{^{11}\text{B}/^{10}\text{B}_{\text{standard}}}\right) - 1\right] \times 1000 \qquad (2)$$

222

223   The reported δ^{11}B is an average of the two analyses, with each representing a fully independent

224   measurement (*i.e.* the two measurements did not share blanks or bracketing standards). Machine

225   stability and accuracy was monitored throughout the analytical session using repeats of NIST SRM 951,

226   as well as boric acid reference materials AE120, AE121 and AE122 that gave δ^{11}B (± 2 s.d.) of -20.19 ±

227   0.20 ‰, 19.60 ± 0.28 ‰, and 39.31 ± 0.28 ‰, that are within error of the gravimetric values from Vogl

228   and Rosner (2012).

229

230   The reproducibilities of the δ^{11}B and [B] measurements were assessed by repeat measurements of

231   TC460 of different total B concentration (11 to 34 ng of B). In order to assess the accuracy of this

232   method, we follow Tipper et al. (2008) and Ni et al. (2010) and use standard addition. To this end,

233   known amounts of NIST SRM 951 standard were mixed with known quantities of TC460. All mixtures

234   were passed through the entire separation and analytical procedure, including aliquots of pure

235   standard and sample. A sodium acetate - acetic acid buffer was added to all 951 boric acid used prior

236   to mixing, to ensure the pH was sufficiently elevated for the column separation procedure (following

237   Foster, 2008). The amount of biogenic silica matrix added to the columns for each mixture was kept

238   constant, so the volume added to the column was altered for each mixture accordingly. Uncertainty

239   in the δ^{11}B calculated for each mixture was determined using a Monte Carlo procedure (n = 1000) in

240 R (R Core Team, 2019) propagating uncertainties, at 95% confidence, in known isotopes ratios (± 0.2

241 ‰), sample concentration (± 6 %) and measured masses (± 0.5 %).

242

243 **3.   Results and Discussion**

244 **3.1 Analytical Technique**

245 **3.1.1. Purification**

246 The Na, Si, and Al concentrations of the matrix fraction of several replicates of the diatom fraction of

247 TC460 are shown in Figure 3a-d. Prior to purification, Na and Si concentrations were consistently

248 around 265 and 114 ppm respectively, whereas Al was more variable at 5-25 ppb. The boron content

249 of these matrix samples in all cases was at blank level. The concentration of these elements in the

250 boron fraction is shown in Figure 3e-g, highlighting that the column procedure was sufficient to

251 concentrate boron and remove Na and Si, which are both present at sub-5 ppb level (*i.e.* at less than

252 0.002 % of matrix concentration). The Al is likely present in the diatom frustule (*e.g.* Koning et al.,

253 2007) and is elevated in the boron fraction compared to the matrix fraction (Figure 3). Diatom-bound

254 Al is likely present as the anion $Al(OH)_4^-$, hence its elevation in the boron fraction. Although this is a

255 detectable level of Al, it is unlikely that this level of contamination will influence the mass fractionation

256 of these samples when measured by MC-ICP-MS (Foster, 2008; Guerrot et al., 2010).

257

258 **3.1.2. Accuracy and Reproducibility**

259 Throughout the duration of this study, a single dissolution of the diatom fraction of TC460 was

260 measured 18 times in separate analyses at various concentrations, in order to assess external

261 reproducibility of this method. Carbonates generally have a reproducibility of ± 0.20 ‰ (2σ) at an

262 analyte concentration of 50 ppb boron using the MC-ICP-MS methods at the University of

263 Southampton (*e.g.* Chalk et al., 2017). The repeated measurements of TC460 gave a reproducibility of

264 ± 0.28 ‰ (2σ) over 18 samples, ranging from 19 ppb to 61 ppb (11 to 34 ng) boron (Figure 4). The

265 insensitivity of $\delta^{11}B$ to the boron concentration analysed confirms that blank contamination during

266 purification is not significant. Figure 4 shows that there is also no correlation between Al content of

267 the boron fraction and measured $\delta^{11}B$, confirming that Al contamination does not influence mass

268 fractionation.

269

270 Figure 4 shows the results of the standard addition experiment, and when the uncertainty in the $\delta^{11}B$

271 of the mixture is considered, it is clear that nearly all the mixtures lie within error of the 1:1 line,

272 indicating that there is a lack of a significant matrix effect when analysing the $\delta^{11}B$ of biogenic silica as

273 described herein. A least-squares linear regression of the mixtures has a slope of 1.01 ± 0.07 and an

274   intercept of -0.15 ± 0.29 ‰, implying the approach is accurate to ± 0.29 ‰, which is remarkably similar

275   to the stated reproducibility of TC460 (± 0.28 ‰ at 2σ).

276

277   B and Si content were determined separately and combined post-analysis in order to estimate the

278   B/Si ratio for each sample and hence the B concentration. The reproducibility of this method was

279   tested using six repeats of the diatom fraction of TC460. The mean of all six measurements is 2.99 ±

280   0.64 ppm; (2σ; Figure 4), implying this multi-stage method of determining the B content of diatoms is

281   precise to ± 20 % at 95% confidence.

282

283   **3.2. Diatom Cultures**

284   **3.2.1.   Boron content of the frustule of *T. weissflogii***

285   The boron content of *T. weissflogii* increases as a function of pH from around ~1 ppm to ~4 ppm over

286   a range of average culture pH from 7.5 to 8.6 (Figure 5; Table 2). While this is lower by an order of

287   magnitude than the limited previous studies of boron in sedimentary diatoms (Ishikawa and

288   Nakamura, 1993), it is similar to boron concentration in the bulk diatom fraction of TC460 (Figure 4D)

289   and to that observed in previous culturing studies of this diatom species (Figure 5; Meija et al., 2013).

290   In detail, however, our concentrations are around 2-3 times lower than Meija et al. (2013), perhaps

291   due to: (i) the different analytical methods used (laser ablation ICP-MS vs. solution here); (ii)

292   differences in cleaning methods; and/or (iii) differences in culturing methodology. Despite the scatter

293   between our treatments (also seen in Meija et al., 2013; Figure 5), a least squares regression through

294   the treatments is significant at the 95% confidence level (y = 2.15x − 15.56, R^2 = 0.46, p = 0.015; Figure

295   5). The cause of this scatter between treatments is not known but a likely contributor is the relatively

296   high variability in the carbonate system which was observed in each treatment due to the growth of

297   the diatoms in this batch culture setup (Figure 2).

298

299   Boron is an essential nutrient for diatoms (Lewin, 1966) and it is likely that boric acid passively diffuses

300   across the cell wall to ensure the diatom cell has sufficient boron to meet its biological needs.

301   However, if boric acid were the sole source of boron for the diatoms measured here we might expect

302   a decrease in boron content as pH increases and external dissolved boric acid concentration declines

303   (Figure 6).

304

305   Several studies note that a number of higher plants have mechanisms for also actively taking up boron,

306   leading to large variations in internal boron concentrations (Pfeffer et al., 2001; Dordas and Brown,

307   2000; Brown et al., 2002). Indeed, on the basis of a similar dataset to that collected here, Meija et al.

308   (2013) suggested that borate is likely transported across the cell wall of *T. weissflogii* as some function

309   of external borate concentration, which shows a positive relationship with external pH (Figure 6). This

310   hypothesis is developed and discussed further in the next section.

311

312   **3.2.2. Frustule δ^{11}B of *T. weissflogii***

313   The δ^{11}B of *T. weissflogii* are isotopically light compared to seawater (39.6 ‰; Foster et al., 2010), with

314   an average value across all treatments of -3.95 ‰ (Table 2). Despite the scatter between treatments,

315   similar to the [B] data, Figure 5 shows that there is a clear relationship between the δ^{11}B of the diatom

316   frustule and pH (R^2 = 0.46, p <0.01), albeit with a negative and relatively shallow slope (y = -2.61x +

317   17.12).

318

319   These results confirm that biogenic silica, free from clay contamination, has a very light boron isotopic

320   composition (Ishikawa and Nakamura, 1993). However, the observed relationship between δ^{11}B in *T.*

321   *weissflogii* and pH is radically different to that which is observed in carbonates (Figure 5), implying a

322   distinctive incorporation mechanism for boron into diatom opal. Much work has been carried out in

323   recent years to show that boron is incorporated in carbonates predominantly as the borate ion with

324   minor, if any, isotopic fractionation (*e.g.* see Branson, 2018 for a review). It is similarly thought that

325   the borate ion is incorporated into opal in an analogous fashion to its incorporation into clays

326   (Ishikawa and Nakamura, 1993; Kolodny and Chaussidon, 2004). However, such a mechanism in

327   isolation would only be able to generate δ^{11}B in opal of ~13 ‰ (at the lowest pH). Given the

328   preponderance of isotopically light diatoms, radiolaria and chert δ^{11}B in the literature (including this

329   study; Kolodony and Chaussidon, 2004; Ishikawa and Nakamura, 1993), it is therefore likely that there

330   is an additional light isotopic fractionation of boron on its incorporation into opal, although its

331   absolute magnitude is currently unknown (Kolodony and Chaussidon, 2004).

332

333   To make their frustules out of biogenic silica, aqueous $Si(OH)_4$ is taken up by the diatom cell via active

334   transport by silicon transporter proteins (Amo and Brzezinski, 1999). Once $Si(OH)_4$ has entered the

335   cell, it accumulates in vacuoles that tend to have a high pH in order to prevent polycondensation of

336    $Si(OH)_4$ at its higher concentration in the vacuole (Vrieling et al., 1999). The accumulated $Si(OH)_4$ is

337   then transported to the silicon deposition vesicle (SDV), which is an acidic compartment where the

338   formation of biogenic silica and the construction of the frustule occurs. Without knowledge of the

339   isotopic fractionation of boron on incorporation into biogenic silica, the interpretation of our new δ^{11}B

340   data is challenging. This difficulty is further increased given that the fluid in the SDV is unlikely to have

341   the same δ^{11}B as external seawater and its relatively acidic pH (~5.5; Meija et al., 2013; Vrieling et al.,

342 1999) is likely to promote polymerisation of $Si(OH)_4$. Nonetheless, the broad similarity between the

343 $\delta^{11}B$ of our cultured *T. weissflogii* with the bulk diatom fraction measured here from sample TC460

344 and the bulk diatom fraction and radiolarian skeleton measured by Ishikawa and Nakamura (1993; ~3

345 ‰), suggests that a large part of the light isotopic composition of biogenic silica is driven by the

346 isotopic fractionation on incorporation rather than "vital effects" relating to the $\delta^{11}B$ and pH of the

347 SDV in the different species and organisms. That being said, the >3‰ range between different pH

348 treatments in *T. weissflogii* and the >10 ‰ difference between our *Chaetoceros* dominated bulk

349 diatom fraction from TC460 and the cultured *T. weissflogii,* as well as the negative relationship

350 between pH and diatom $\delta^{11}B$ (Figure 5), argue against a simple two-step model involving borate ion

351 incorporation from seawater with a fixed isotopic fractionation.

352

353 The $\delta^{11}B$ of the fluid from which our *T. weissflogii* precipitated their frustules can be calculated if

354 we assume the pH in the SDV of our *T. weissflogii* is 5.5 across all our treatments (Mejia et al.,

355 2013). Given that at this pH the $\delta^{11}B$ of borate is ~13 ‰, the isotopic composition of this fluid is

356 lighter than seawater, even if we assume an arbitrary −10 ‰ isotopic fractionation on

357 incorporation (blue circles in Figure 7a). Furthermore, the $\delta^{11}B$ of the SDV fluid is inversely

358 correlated with the $\delta^{11}B$ of either dissolved borate or dissolved boric acid (Figure 7a).

359 As discussed above and illustrated schematically in Figure 8, Mejia et al. (2013) suggested that

360 there are two sources of boron in a diatom cell: (i) passively diffused and isotopically heavy boric

361 acid; and (ii) actively transported isotopically light borate ion (see Figure 8). Assuming that: (a) no

362 additional fractionation occurs during uptake and diffusion; and (b) only the borate ion is

363 incorporated into the frustule, we can calculate the relative contribution of these two sources of

364 boron as a function of external pH (Figure 7b). This treatment shows that the relative

365 concentration of borate derived boron in the SDV fluid increases as external pH increases, though

366 the absolute values here are a function of the magnitude of the isotopic fractionation on

367 incorporation, so we only have confidence in the trends shown in Figure 7b. Nonetheless, given

368 that the dissolved boric acid concentration decreases and dissolved borate increases as pH is

369 increased (Figure 6), this is perhaps not surprising.

370 While this finding is entirely compatible with the trend of increasing boron content of *T.*

371 *weissflogii* observed as pH increases (Figure 5), an added complication is that at pH ~5.5 the

372 concentration of borate ion in the SDV is likely to be relatively low (Figure 6). However, the

373 timescales required to reach equilibrium in the boron system are short (e.g. around 95 μs; Zeebe

374   et al., 2001), meaning that any aqueous borate incorporated into the frustule would be

375   immediately replenished to its equilibrium value by conversion from the more abundant boric

376   acid. Although relevant partition coefficients are likely to be different, a similar process ensures

377   the quantitative removal of boron from pH <7 solutions by the Amberlite 743 anion exchange

378   resin used for boron purification prior to analysis by MC-ICPMS (see above; Lemarchand et al.,

379   2002).

380   Active bicarbonate ion uptake accounts for a substantial amount of the carbon fixed by phytoplankton

381   (e.g. Tortell et al., 2006). As a result, Mejía et al. (2013) proposed that the enrichment of borate ion

382   into the SDV of *T. weissflogii* and *T. pseudonana* was the result of the active co-transport of borate ion

383   with bicarbonate ion by bicarbonate transporter proteins. Borate is transported because of its similar

384   charge and size to HCO_3^- and the phylogenetic similarity between bicarbonate and borate transporters

385   (Mejía et al., 2013). In our model, as external borate ion concentration increases, the borate leak into

386   the diatom cell is also increased. An additional factor is HCO_3^- transport, which may be proportionally

387   up-regulated as external CO_2 content decreases (and external pH increases) in order to provide the

388   diatom cell with sufficient carbon (Mejía et al., 2013). This may therefore offer a way of driving an

389   elevation of the borate content of the SDV as pH increases (Mejía et al., 2013). Regardless of the exact

390   mechanism, an SDV fluid with an inverse relationship between $\delta^{11}B$ and pH is required to explain the

391    $\delta^{11}B$ of the *T. weissflogii* frustule measured here. A simple model whereby external borate ion is an

392   increasingly important contributor to the boron in the SDV as pH increases is able to explain the

393   observed dependency of boron content and $\delta^{11}B$ on pH. However, a more complete model of the

394   boron systematics in diatom opal requires a better understanding of isotopic fractionation on

395   incorporation of boron into biogenic silica, the environmental controls on this fractionation and the

396   nature of the partitioning of boron within the diatom cell and into biogenic silica.

397

398   **3.2.3.   Boron-based pH proxies in diatom opal**

399   The $\delta^{11}B$-pH and B-pH relationships derived here for *T. weissflogii* potentially offer two independent

400   means to reconstruct the past pH of seawater, particularly in those regions key for CO_2 and heat

401   exchange where foraminifera are largely absent (e.g. at high latitudes). However, the current

402   calibrations (Figure 5) are relatively uncertain, which may preclude their application to some

403   situations. For instance, recasting the $\delta^{11}B$-pH relationship in terms of $\delta^{11}B$ as the dependent variable

404   and using a regression method that accounts for uncertainty in X and Y variables (SIMEX; Carroll et al.,

405   1996) gives the calculated residual pH of the regression as ± 0.28 pH units. For the [B] vs. pH

406   relationship, this uncertainty is ± 0.36 pH units. At typical surface ocean conditions, such a variability

407 in pH would translate to seawater pCO$_2$ variability of up to ca. ± 250 ppm. Although encouraging, this
408 treatment suggests that additional work is needed before the relationship between δ^{11}B and boron
409 content of diatom opal and seawater pH is a sufficiently precise proxy for a fully quantitative past
410 ocean pH. In particular, future culturing efforts should aim to more carefully control the pH of the
411 culture media. This could be achieved by either using larger volume dilute batch cultures, by
412 harvesting the diatoms earlier in the experiment prior to any significant drift in the carbonate system,
413 and/or by using a more robust steady-state chemostat method (e.g. Leonardos and Geider, 2005).

414

415 **4. Conclusions**

416 In the first study of its kind, we use a modified version of the carbonate boron purification technique
417 of Foster (2008) to show that the δ^{11}B of *T. weissflogii* opal is pH sensitive but isotopically light (-3.95
418 ‰ on average) and has an inverse relationship with external seawater pH. Using a novel ICP-MS
419 method we also show that the boron content of *T. weissflogii* opal increases with increasing pH,
420 supporting the only other study investigating boron in diatoms (Mejía et al., 2013). This suggests that
421 more borate is incorporated into the diatom frustule as the dissolved borate abundance increases
422 with external pH. A simple model is presented, based on Mejía et al. (2013), which implies both of
423 these findings could be due to there being two distinct sources of the boron in the SDV: external boric
424 acid and external borate ion, with the balance of each source changing with external pH. While these
425 results are encouraging, suggesting that the boron proxies in diatom opal may hold considerable
426 promise as a tracer of past ocean pH, more work is needed to fully understand the boron systematics
427 of diatom opal. In particular, there is an urgent need to place boron in opal on a firmer grounding with
428 precipitation experiments in the laboratory at controlled pH to determine the magnitude of boron
429 isotopic fractionation on boron incorporation into opal as well as the dependence of this fractionation
430 on other environmental factors.

431

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438

439 **Author contribution**

440 GLF, HKD, AJP and CMM conceived and designed the study and it was carried by HKD and NF (aided

441 by AJP, CMM and GLF). GEAS aided HKD in sample preparation and MPH carried out the carbonate

442 system measurements of the culture media. GLF and HKD produced the first draft and all authors

443 contributed to the writing of the study.

444

445 **Competing interests**

446 The authors declare that they have no conflict of interest.

447

448 **Code/Data availability**

449 The data generated in this study is tabulated herein. For any additional data please contact the

450 corresponding author.

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459   **Figure Captions**

460   **Figure 1**. Diatom growth rate and cell size as a function of pH labelled according to CO_2 treatment.

461   Linear least squares regressions, including R^2 and p-values are also shown.

462   **Figure 2**: Each culture treatment labelled according to target pCO_2 and showing the evolution in

463   the culture media through the experiment. All treatments exhibit changes in DIC due to diatom

464   growth balanced with the input of CO_2. The higher pCO_2, the more DIC increases towards the end

465   of the experiment.

466   **Figure 3**: (a-d) Concentration of Na, Si, Al and B in the Matrix Fraction by ICP-MS. These analyses

467   suggest blank levels of B are present in the matrix washed off the Amberlite IRA 743 resin-based

468   column. (e-f) Concentration of the Na, Si and Al in the boron fraction indicating blank levels of Na

469   (ca. 1.7 ppb) and Si (ca. 1.9 ppb) and a higher concentration of Al (ca. 68 ppb) are present.

470   **Figure 4:** (A) The reproducibility of the TC460 diatom core catcher in-house standard. Samples of

471   different concentration (~10 to ~30 ng B) lie within error of the mean (5.98 ‰ ± 0.28 ‰, 2σ). This

472   compares well to carbonates (2σ = 0.20 ‰). (B) Aluminium concentration of the B fraction from

473   TC460 (as ppb of the solution analysed for $\delta^{11}B$) shows no correlation with $\delta^{11}B$, likely suggesting

474   there is no significant effect on mass fractionation for this level of Al. (C) The results of the standard

475   addition experiment. The blue line is a least squares regression between the measured $\delta^{11}B$ of each

476   mixture (green circles) and the calculated $\delta^{11}B$ of that mixture given known end-member values (end

477   members shown as blue circles). R^2 = 0.97, p < 0.0001, slope = 1.01 ± 0.07 and intercept = -0.15 ±

478   0.29. 1:1 line is shown as a black line and dotted blue lines show the 95% confidence limit of the

479   regression. Note that the end members were not used in the regression. (D) B content in ppm of six

480   repeat samples of the diatom fraction of TC460. The black line indicates the mean value and the grey

481   lines show 2σ, of 2.99 ± 0.64 ppm.

482   **Figure 5**: (A) $\delta^{11}B$ of *T. weissflogii* diatom opal plotted against aqueous borate, labelled according to

483   pCO_2 treatment. Also shown are published deep sea coral *Desmophyllum dianthus* (Anagnostou et

484   al., 2012) and foraminifera $\delta^{11}B$ (*Globigerinoides ruber* and *Orbulina universa*; Henehan et al., 2013;

485   Henehan et al., 2016, respectively). Least squares regression lines are also shown. Error bars on $\delta^{11}B$

486   borate are shown at 95% level of confidence and relate to the drift in experimental conditions. (B)

487   *T. weissflogii* opal $\delta^{11}B$ against pH of each treatment demonstrating a statistically significant negative

488   relationship. Diatom data is labelled according to pCO_2 treatment. (C) Boron content of cultured *T.*

489   *weissflogii* diatom opal as a function of pH (using left-axis), labelled according to pCO_2. A least

490   squares regression with 95% confidence interval is also shown. In grey (and using the right-hand

491   axis) are data for *T. weissflogii* from Mejia et al. (2013). Note how both studies show an increase in

492   boron content with increasing pH, but absolute values differ by a factor of 2-3. Uncertainty in all

493   points is shown at the 95% confidence level. In some cases, the error bars are smaller than the

494   symbols.

495   **Figure 6**: Plots describing (A) the pH-dependent relationship between the abundance of aqueous

496   boron species, and (B) the isotopic fractionation observed between boric acid ($B(OH)_3$; red) and

497   borate ($B(OH)_4^-$; blue) at T = 25 $^\circ$C and S = 35.

498   **Figure 7**: (A) Back-calculated $\delta^{11}B$ of the silica deposition vesicle (SDV), and (B) the fraction of boron

499   in the SDV that is derived from external borate. In (A) the diatom $\delta^{11}B$ data are shown as grey circles

500   and the calculated $\delta^{11}B$ of the SDV as blue circles. Included in this model is an arbitrary -10 ‰

501   fractionation between the $\delta^{11}B$ of the SDV and the opal precipitated. The fraction of borate in the

502   SDV in (B) is a function of this assumption so these absolute values should be taken as illustrative

503   only.

504   **Figure 8**. Schematic of the model described herein for boron uptake by *T. weissflogii*. The speciation

505   behaviour and isotopic composition of boron is also shown in the insert, with the aqueous species

506   colour coded (red = boric acid, blue = borate ion). Seawater boric acid diffuses into the diatom cell

507   and the borate ion is actively transported, with HCO_3^-. While it remains unclear how boron enters

508   the silica deposition vesicle, once inside it respeciates into borate ion and boric acid, with the borate

509   ion being incorporated into the frustule. The isotopic composition of internal boron is a function of

510   external pH, which sets the isotopic composition of the incoming species, and the balance between

511   active borate ion transport and passive boric acid diffusion. The compartments are colour coded

512   according to approximate pH (scale on the right).

513

514

515

Tables

517

Treatment	pCO$_2$ (ppm)	2σ	pH	2σ	DIC (μM)	2σ	HCO$_3^-$ (μM)	2σ	Growth rate (d^{-1})
200	125	8	8.53	0.73	1925	61	1091	59	1.03
280	244	73	8.25	0.41	2165	113	1521	260	1.03
400	267	28	8.25	0.44	2400	115	1728	107	0.96
800	809	62	7.83	0.24	2525	56	2206	69	1.01
1600	2117	40	7.48	0.08	2791	21	2628	22	1.01

518   *Table 1: Mean carbonate system parameters experienced under the average growth*

519   *conditions as calculated for each culture treatment on the basis of the number of cells*

520   *grown in each 24-hour period of the batch experiment.*

521

Treatment	pH (Total scale)	pH 2σ	δ^{11}B	δ^{11}B 2σ	δ^{11}B sw borate	[B] ppm
200	8.55	0.63	-5.51	0.21	24.20	3.15
200	8.54	0.62	-5.40	0.21	24.00	2.81
280	8.27	0.35	-5.05	0.20	20.00	3.72
280	8.18	0.25	-5.66	0.21	18.80	0.93
280	8.30	0.42	-5.79	0.21	20.50	1.04
400	8.26	0.38	-3.64	0.20	19.90	3.37
400	8.24	0.36	-3.57	0.21	19.60	1.26
400	8.25	0.36	-2.41	0.21	19.70	2.68
800	7.85	0.22	-2.93	0.19	15.40	NA
800	7.82	0.18	-2.80	0.22	15.20	0.78
800	7.82	0.20	-3.08	0.21	15.20	1.11
1600	7.48	0.06	-1.94	0.20	13.30	0.74
1600	7.48	0.07	-3.62	0.21	13.30	0.91

522   *Table 2. Treatment name and pH with δ^{11}B and [B] for cultured T. weissflogii.*

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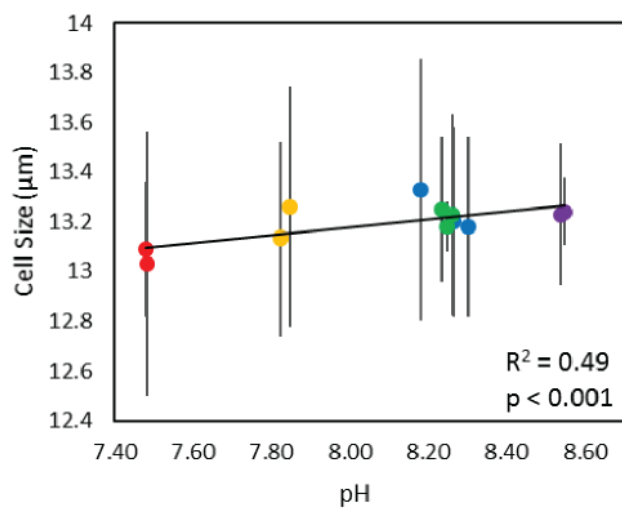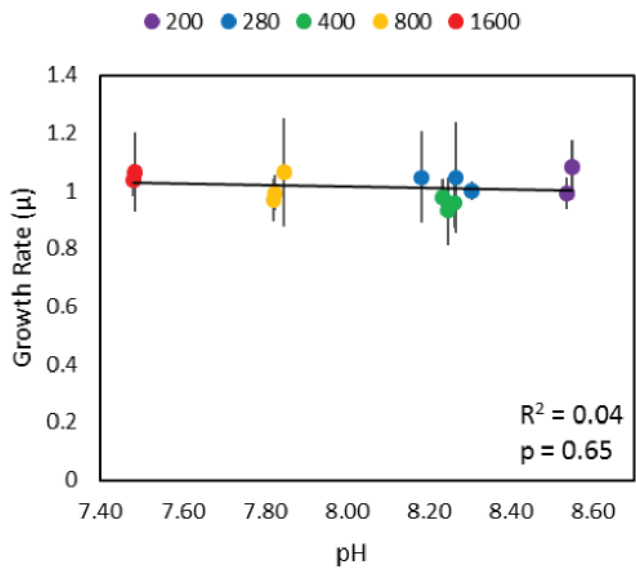
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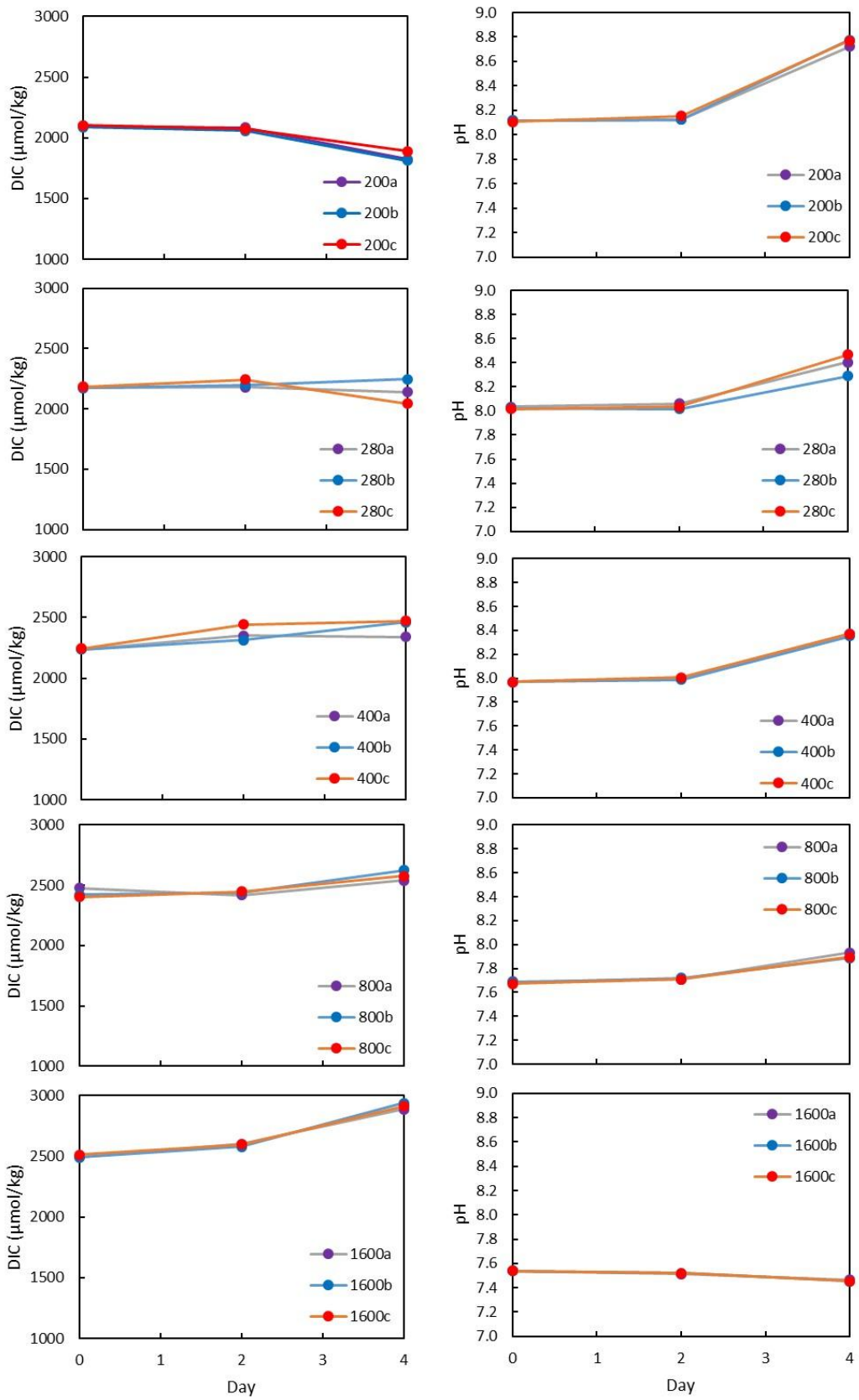
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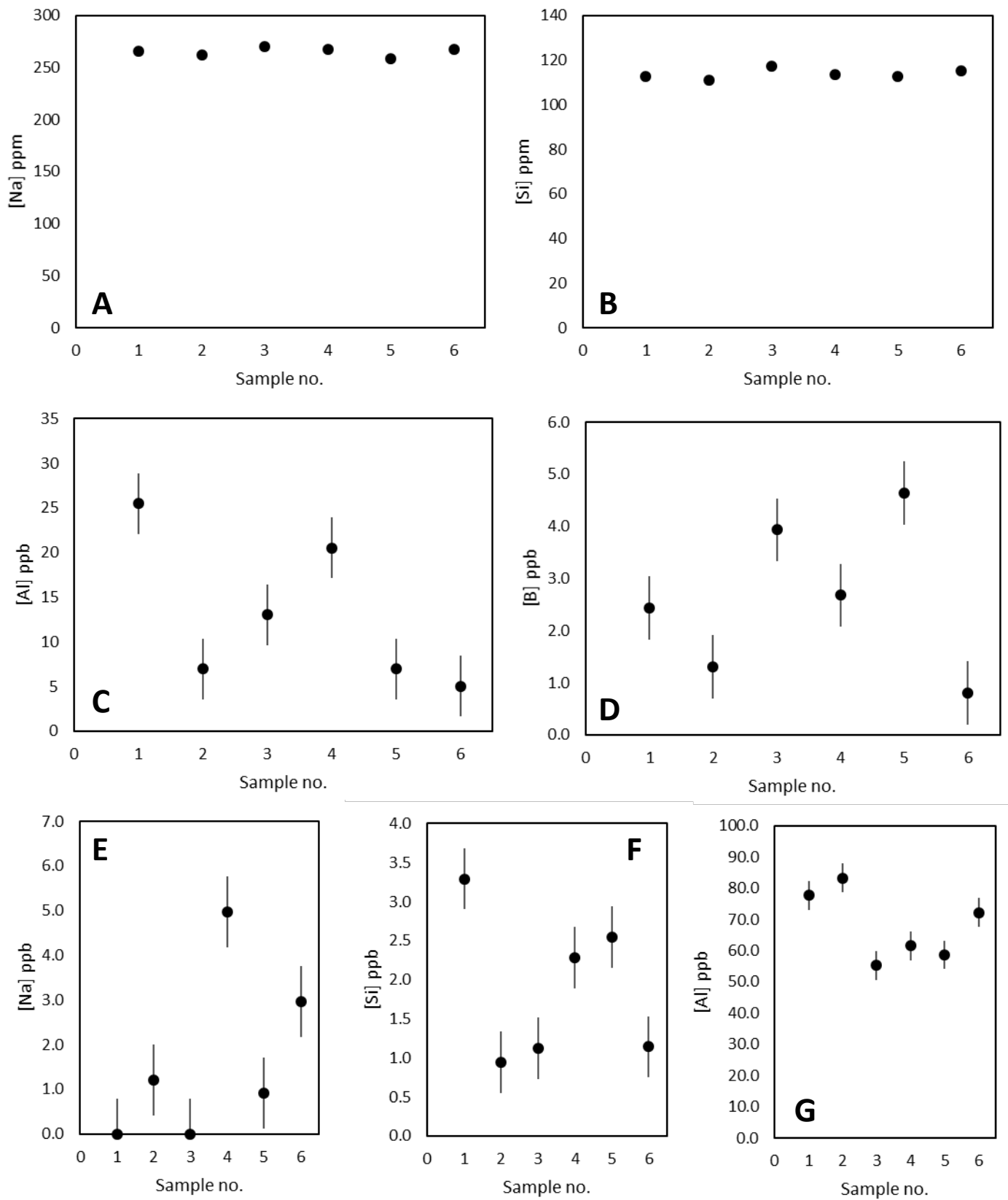
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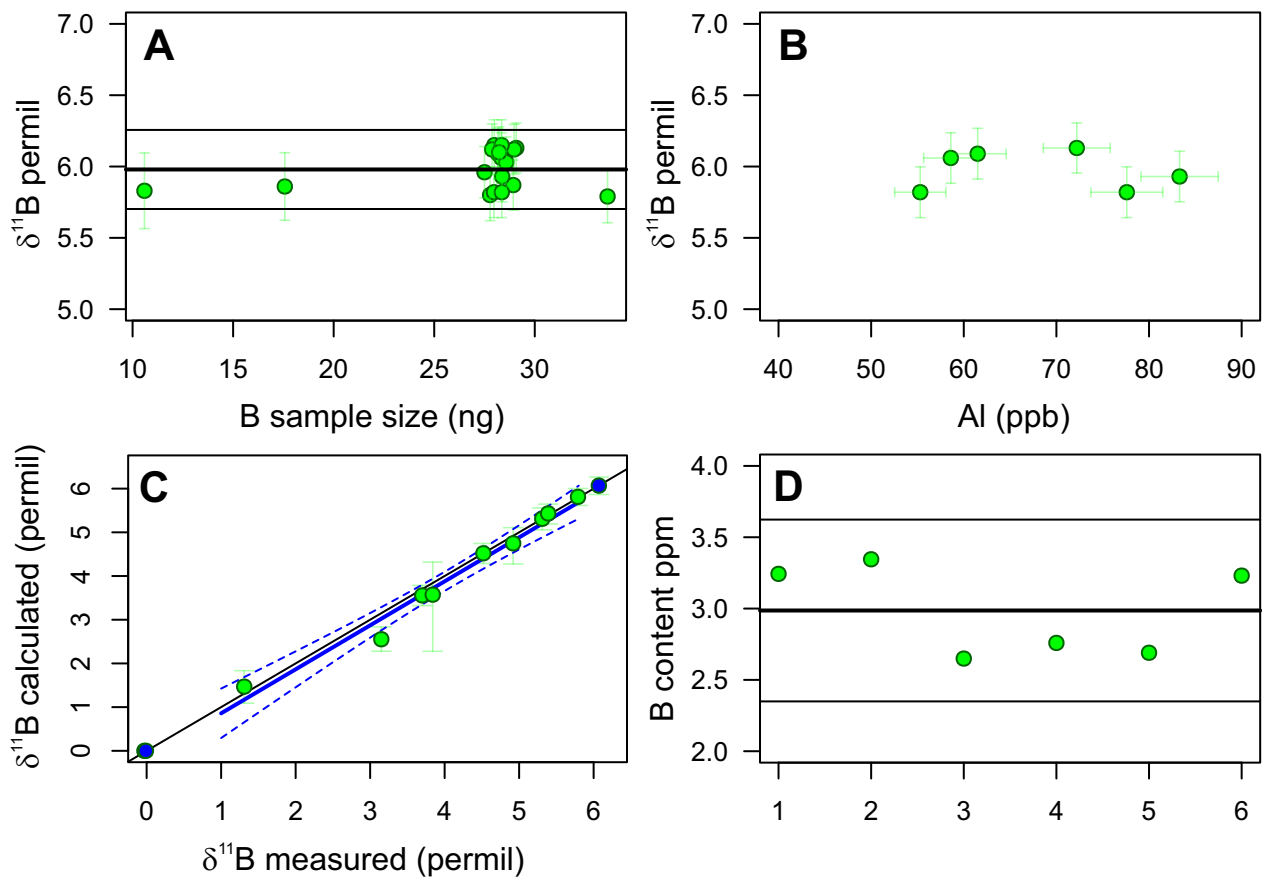
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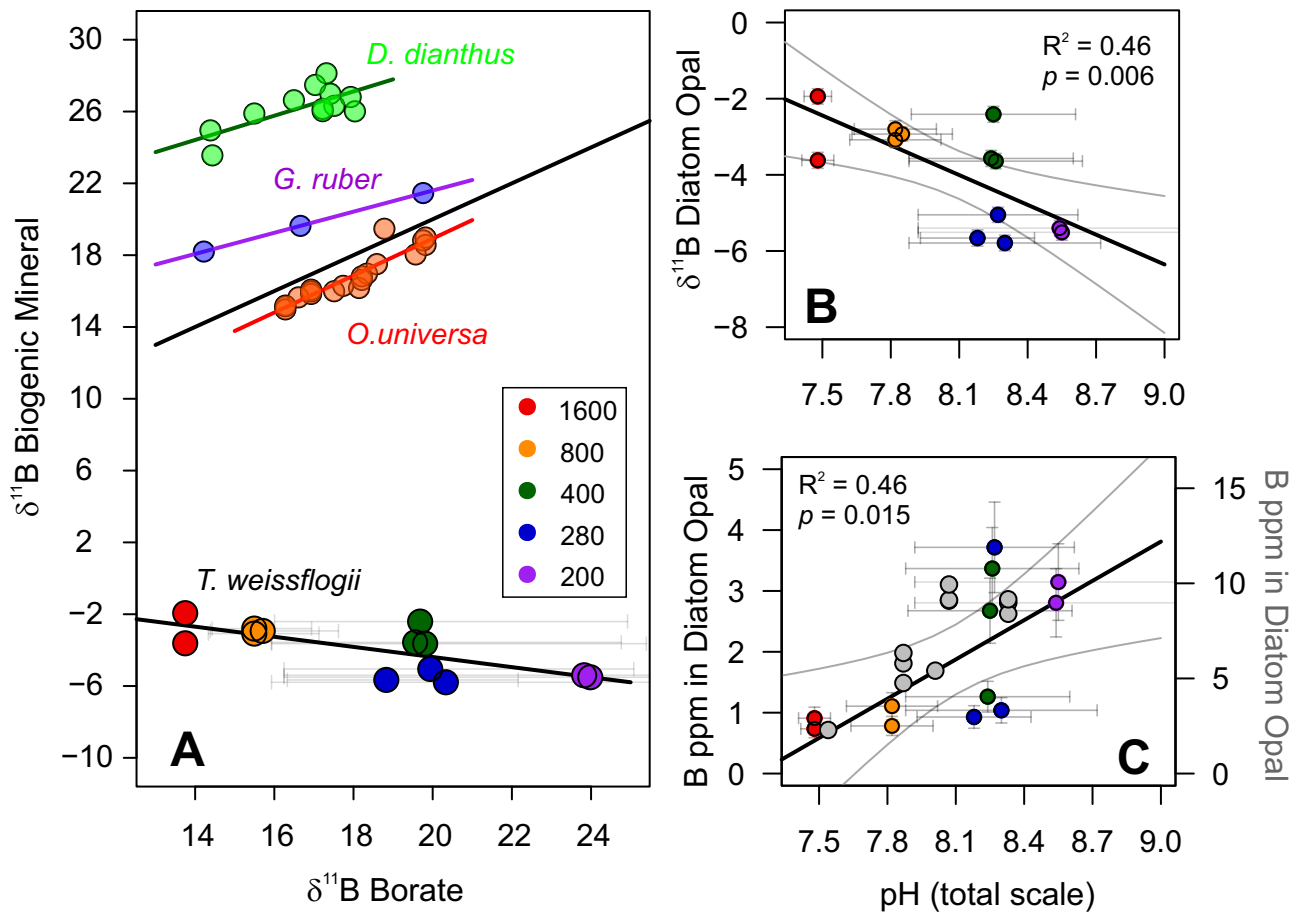
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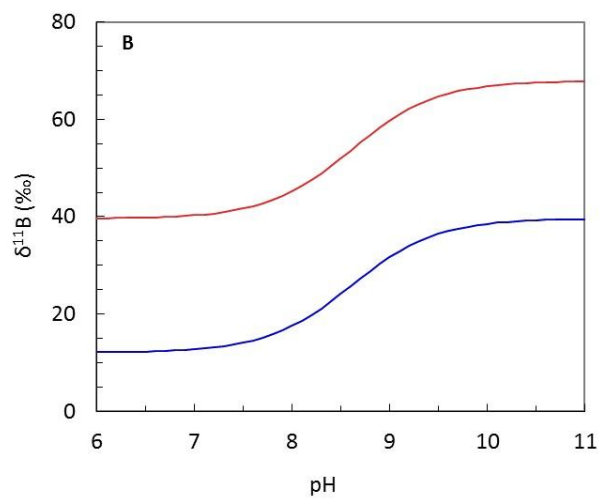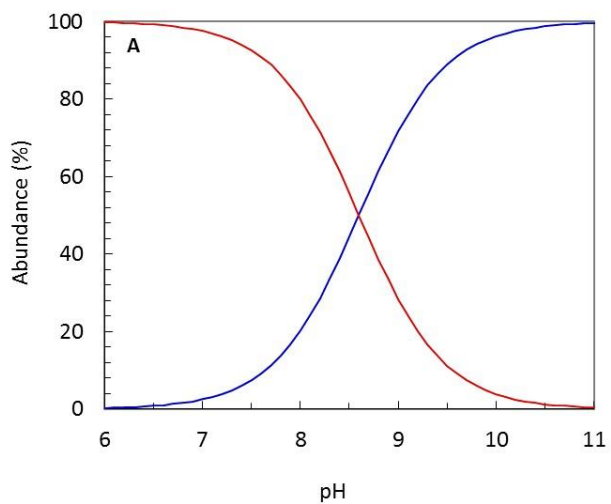
Figure 6.

Figure 7

Figure 8.