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1   **Impacts of pH and $[CO_3^{2-}]$ on the incorporation of Zn in foraminiferal calcite**

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11

12   **Abstract**

13   The trace elemental composition of foraminiferal shell calcite is known to reflect the environment in

14   which the shell was precipitated. Whereas conservative elements incorporated in foraminiferal shell

15   carbonate reflect factors such as temperature (Mg), carbonate chemistry (B) and salinity (Na), the

16   nutrient type elements (Ba, Cd, and possibly Zn) are useful tools to reconstruct biogeochemical cycling

17   and past ocean circulation. Still also nutrient-type elements will be most likely influenced by factors

18   other than their relative concentrations. Culturing benthic foraminifera under controlled carbonate

19   chemistry conditions allows for disentanglement of impacts of different parameters of the carbon system

20   on the elemental composition of foraminiferal calcite. Here we show that zinc incorporation in cultured

21   specimens of the benthic foraminifer *Ammonia tepida* is correlated to changes in carbonate ion

22   concentration ($[CO_3^{2-}]$). By modeling activities of different chemical species of Zn in seawater over a

23   range of $[CO_3^{2-}]$, we suggest that Zn^{2+}, rather than other relatively abundant Zn-species (e.g. $ZnCO_3^0$

24   and $ZnHCO_3^+$) is taken up during biomineralization. Our results suggest that foraminiferal Zn/Ca might

25   be especially useful when combined with other $[CO_3^{2-}]$ proxies, enabling reconstruction of past seawater

26   element concentrations. Conversely, when the nutrient-type element concentrations are known,

27   incorporation of Zn in foraminiferal shells can be used to reconstruct past sea water carbon speciation.

1. Introduction

From the active carbon cycle more than 95% is present in dissolved inorganic form in seawater (Zeebe and Wolf-Gladrow, 2001). Over geological times, changes in carbon speciation determine the amount of CO_2 in the atmosphere and studies of past carbon cycling has provided important insights in the relation between climate and carbon cycling. Past marine inorganic carbon cycling can only be assessed by independently reconstructing at least two individual parameters of the so-called CO_2 system (e.g. pH, ΣCO_2, alkalinity, $[CO_3^{2-}]$: see Zeebe and Wolf-Gladrow, 2001 for details). The interdependency of these parameters can subsequently be used to reconstruct the complete inorganic carbon system over geological timescales using any combination of the two reconstructed parameters. Combining reconstructed seawater pH and alkalinity (e.g. Hönisch and Hemming, 2005), for example, has resulted in reconstruction of Pleistocene dissolved CO_2 concentrations, thereby allowing estimates of atmospheric CO_2 levels (Hönisch et al., 2009). Accuracy and precision of these reconstructions depend on the quality and the extent to which the applied proxies are truly decoupled. The chemical composition of calcite shells produced by foraminifera is widely used for reconstructing past climate parameters. Besides functioning as a paleothermometer (e.g. using calcitic Mg/Ca; Nürnberg et al., 1996; Nürnberg, 2015; Evans et al., 2016), element concentrations (e.g. B/Ca) and stable isotope ratios (e.g. $\delta^{11}B$) in foraminiferal shells can also reflect different parameters of the inorganic carbon system, including pH and $[CO_3^{2-}]$ (Sanyal et al., 1996; Raitzsch et al., 2011). The co-variation of carbon system parameters in nature often limits field calibrations and core top studies, since this obscures the impact of a single parameter on foraminiferal calcite chemistry. Moreover, such calibrations are often hampered in their application due to additional and/or unknown impacts of other parameters of the inorganic carbon system, environmental parameters (e.g. temperature, salinity) and vital effects (e.g. growth rate). For instance, application of the reported correlation between foraminiferal B/Ca and $[CO_3^{2-}]$ (Yu and Elderfield, 2007; Yu et al., 2010; Rae et al., 2011) may be complicated by additional controls on boron incorporation by changes in seawater pH (Hemming and Hanson, 1992), DIC and/or $[Ca^{2+}]$ (Uchikawa et al., 2015), temperature and/or salinity (Allen et al., 2011). Experiments that separately assess impacts of different environmental parameters are therefore necessary to improve their application.

55 To study the isolated impacts of carbon system parameters on foraminiferal calcite chemistry, laboratory

56 experiments where foraminifera are cultured under controlled conditions were designed (Spero et al.,

57 1997; Keul et al., 2013b). In such experiments, the isolated impact of e.g. pH is studied, when comparing

58 foraminifera grown under conditions with variable versus stable pH. Such an approach was also applied

59 to determine the individual impacts of pH and $[CO_3^{2-}]$ on U incorporation in foraminiferal calcite, which

60 was previously found to correlate with both pH and $[CO_3^{2-}]$ (Russell et al., 2004; Raitzsch et al., 2011).

61 These experiments suggested that carbonate ion concentration rather than other parameters of the

62 inorganic carbon system, controls foraminiferal U/Ca and thereby highlight its potential as an

63 independent $[CO_3^{2-}]$ proxy (Keul et al., 2013a).

64 Inorganic precipitation experiments previously showed that Zn adsorption onto calcite depends on

65 solution pH (Zachara et al., 1991). However, core top studies (Marchitto et al., 2000; Marchitto et al.,

66 2002) suggested that both Cd/Ca and Zn/Ca in foraminiferal shell carbonate covary with calcite

67 saturation (ΔCO_3^{2-}) and might thus provide a paleoceanographic tracer for deep water masses. At the

68 same time due to their nutrient like behavior, Cd and Zn have the potential to be used in concert to

69 reconstruct small changes in nutrient concentrations in thermocline waters and carbonate saturation state

70 (Bryan and Marchitto, 2010). Since these parameters are difficult to separate in the field we here

71 calibrate incorporation of zinc (Zn) in foraminiferal shell calcite as a function of individually varied

72 seawater pH and $[CO_3^{2-}]$. We developed an experimental set-up in which these parameters are varied

73 independently, with the newly formed calcite being analyzed for Zn/Ca using LA-ICP-MS. Response to

74 pH and $[CO_3^{2-}]$ were evaluated against inorganic chemical speciation of Zn.

2. Methods

2.1 Foraminifera collection

Between January and April 2013, surface sediment samples were collected during low tide at an intertidal mudflat near Texel, the Netherlands (coordinates: 53°0'21.4"N; 4°45'8.6"E). Samples were sieved over a 1 mm screen to remove the benthic macrofauna. Sediment was stored in small aquaria with seawater collected at the sampling site, thus providing a stock of foraminifera for culture experiments. Prior to the culturing experiments in summer 2013, material from the stock sediment was sieved over a 150 μm screen to concentrate foraminiferal specimens of the species *Ammonia tepida*. From the resulting >150 μm size fraction, living specimens characterized by yellow cytoplasm and pseudopodial activity, were selected for the culturing experiments.

2.2 Seawater preparation

Surface water (salinity = 35.2) from the North Atlantic was filtered (0.2 μm pore-size) and used as a basis for production of approximately 10 L of stock culture media. Since natural Zn/Ca of surface seawater is relatively low (on average 4.4 µmol/mol; Boyle, 1981; Marchitto et al., 2002), Zn concentration of the culture water was increased by adding 2.000 µmol of Zn ICP standard (1000 mg/L, $Zn(NO_3)_2$ in HNO_3 2-3% CentiPUR) to the stock solution. This increases the Zn/Ca of the culture water approximately 15 times compared to average open ocean seawater [Zn]. Increasing the element to calcium ratio of surface seawater [Zn] below 0.1 mg/L does not influence survival and chamber addition rates of the miliolid foraminifera *Pseudotriloculina rotunda (Nardelli et al., 2013)*. For *Ammonia tepida*, increasing the heavy metal element (e.g. Ni) to calcium ratio of surface seawater less than 20 times does not influence growth and morphology (Munsel et al., 2010). The pH of this stock solution was increased to 8.1 by adding a small volume (~5 ml) of 0.1 M NaOH. Subsamples were taken to determine DIC and alkalinity of the stock solution, which was stored in a 10 L Nalgene container at 10°C and placed in the dark. Eight volumes of 750 ml of this stock solution were manipulated with one of two different carbonate system treatments, resulting in four 750 ml bottles per seawater treatment. The advantage of using a single stock solution for all treatments is that they will have identical seawater Zn/Ca.

2.2.1 Carbonate and pH manipulation

In the first treatment, the pH stable treatment, seawater was manipulated to derive media with varying $[CO_3^{2-}]$, while keeping a similar pH. This was accomplished by addition of pre-defined amounts of $NaHCO_3$ (30-265 mg) to four 750 ml bottles filled with seawater from the stock solution. The experimental range included one treatment with $[CO_3^{2-}]$ lower than the stock solution and, therefore, seawater was acidified with HCl and bubbled with nitrogen to lower DIC by CO_2 outgassing. The [DIC] was then increased to the target value by addition of $NaHCO_3$. For all four treatments, added volumes of [DIC] were estimated using CO2SYS software (Pierrot et al., 2006) to relate the inorganic carbon chemistry to target atmospheric CO_2 concentrations (i.e. 180, 760, 1400 and 2000 ppm), representing pre-industrial, predicted concentration for the end of 21st century, and two extreme greenhouse conditions. Pre-mixed gasses with exactly these concentrations were subsequently used during the incubations.

For the second treatment, the acidification experiment, four 750 ml-bottles were filled with seawater from the stock solution and addition of a small volume (~0.5 ml) 0.1M HCl or 0.1M NaOH to obtain media with a range of pH values, whilst keeping a relatively constant [DIC]. Bottles with teflon lining were used to store both sets of treatment solutions without headspace at 7 °C in the dark until start of the culture experiments.

2.3 Culture set-up

Four air tight incubators (each with a volume of approximately 10 L) were installed in a climate chamber with an ambient, constant temperature of 25°C. Incubators were placed in a cabinet, with a day-night cycle of 12 hours:12 hours (300 lux) and connected to four different CO_2 enriched air premixes corresponding with the equilibrium pCO$_2$ of the treatments (180, 760, 1400 and 2000 ppm CO$_2$). Prior to entering the 10 L incubators, gas was bubbled through a vessel with deionized water to humidify the gasses flowing into the incubators, minimizing evaporation of the culture media. In each of the four incubators, two 500 ml-bottles containing stock media from both seawater manipulations were stored under these conditions for 4 weeks prior to the start of the experiment to serve as pre-equilibrated stock solutions to be used to replace the foraminiferal culture media. At the start of the culture experiments,

131   Petri dishes containing approximately 20 ml of medium and 5-6 foraminiferal specimens from either

132   one of the stocks were placed (Fig. 1) in each incubator, consequently resulting in 10-17 foraminifera

133   per seawater medium per CO_2 treatment.

134   At the start of the experiment, pictures of individual foraminifera were taken with a DFC420 Leica

135   camera coupled to a M165C Leica microscope to determine amount of chambers per individual at the

136   beginning of the experiment. We were able to identify and recognize our specimens since there were

137   only 5-6 individuals per Petri dish, and every specimen has unique characteristics for recognition

138   (chamber shape, small (coiling) malformations, test diameter, etc.). Twice a week the foraminifera were

139   fed with concentrated *Dunaliella salina* cells. The cultured algal cells were centrifuged three times for

140   2 minutes at 2000 rpm, after which the solvent was siphoned off and replace with de-ionzed water after

141   every centrifuge step in order to discard the algae culture media. The resulting *Dunaliella* cells were

142   freeze-dried to remove any remaining solvent and diluted in 1 ml of treatment water, resulting in 8

143   different batches of 1 ml feeding solution. Once a week the culture media were replaced with new

144   equilibrated seawater from the stocks kept in the same incubator. Twice a week, subsamples were taken

145   from the culture water stocks for pH and once every two weeks for DIC and alkalinity analyses. Every

146   time the incubator was opened, the CO_2 premix flow rate was increased from 2L/ hour to 60L/ hour for

147   approximately 60 minutes to quickly replace the air within the incubators, thereby minimizing

148   fluctuations in atmospheric pCO$_2$ during the culturing experiment. After 6 weeks, all specimens were

149   harvested and photographed again to determine the number of newly grown chambers for each

150   treatment.

151

152   **2.4 Analytical methods**

153   **2.4.1 Carbonate system parameters**

154   pH of the different treatments was measured twice a week using a pH meter (pH110, VWR), to assess

155   the stability of the carbonate system during the experiment. The stock seawater (North Atlantic surface

156   seawater) was subsampled twice for pH, TA and DIC measurements, prior to culture treatment

157   manipulations. Subsamples of the culture media were taken in duplicate to determine DIC

158   concentrations and total alkalinity (TA) twice a week, starting after the pre-incubation period of four

159   weeks. In order to analyze the DIC of the different treatments, subsamples were collected in headspace

160   vials containing a saturated $HgCl_2$ solution (10µl $HgCl_2$/10 ml sample). DIC measurements were

161   performed on an autoanalyzer TRAACS 800 spectrometric system (Stoll et al., 2001). These analyses

162   require only a small amount of sample, while yielding high accuracy (±2 µmol/kg) and precision (±1.5

163   µmol/kg). Subsamples for TA analysis were collected in 50 ml Falcon tubes and stored after addition of

164   a small volume of saturated $HgCl_2$ solution (10µl $HgCl_2$/10 ml sample). TA was determined using the

165   standard operating procedure for open cell potentiometric titration (Dickson et al., 2007; SOP 3b), using

166   an automatic titrator (Metrohm 888, Titrando), a high accuracy burette (± 0.001 mL), a thermostated

167   reaction vessel (25 ^{0}C) and a combination pH glass electrode (Metrohm 6.0259.100). TA values were

168   calculated by a non-linear least-squares fit to the titration data (Dickson et al., 2007, SOP 3b) in a

169   custom-made script in the open source programming framework R. Quality assurance involved regular

170   analysis of Certified Reference Materials (CRM) obtained from the Scripps Institution of Oceanography

171   (Dickson et al., 2003). The average relative external precision of the TA measurements is 4.64%.

172   Other carbonate system parameters (including $[CO_3^{2-}]$) were calculated using the average DIC and TA

173   values per treatment and the software CO2SYS v2.1, adapted to Excel by Pierrot et al. (2006). With the

174   equilibrium constants for K1 and K2 of Mehrbach et al. (1973), refitted by Dickson and Millero (1987),

175   we were able to estimate $[CO_3^{2-}]$ and $\Omega_{CALCITE}$ and compare calculated atmospheric CO_2 and pH values

176   to the premix and measured values.

177

178   2.4.2 Seawater zinc and calcium analysis using SF-ICP-MS

179   Culture media were subsampled (1 mL), and diluted 300 times with 0.1M HNO_3 before measuring the

180   media's minor and major element compositions in triplicate using an Element-2 sector field double

181   focusing Inductive Coupled Mass spectrometry (SF-ICP-MS; Thermo Scientific, Bremen, Germany)

182   run in medium-resolution mode (m/Δm=3000) to avoid the effect of mass interferences (e.g.

183    $^{40}Ca+^{26}Mg$). The inlet of the mass spectrometer was equipped with a teflon microflow nebulizer and a

184   Peltier cooled spray chamber to maximize stability of the signal. Calcium concentrations were calculated

185   using an external calibration method with seawater matrix-matched standards. External precisions

186   (RSD) are 6% for ^{25}Mg, ^{88}Sr and ^{43}Ca. We obtained an average [Mg^{2+}] of 47±2 mmol/kg, [Sr^{2+}] of 50±6

187   µmol/kg and [Ca^{2+}] 8.4±0.3 mmol/kg for all treatments (Table 2).

188   Zn concentrations were determined by pre-concentration over an in-house build ion exchange column

189   (volume approximately 70µl) containing NOBIAS-chelate P1 resin (following Biller and Bruland,

190   2012). Subsamples of 30 ml were adjusted prior to pre-concentration to a pH of 6.5 ±0.2 using a 3.7M

191   ammonium acetate buffer. Each column was conditioned with 0.05M ammonium acetate buffer for 1

192   minute after which the seawater was loaded for approximately 20 minutes. After loading the column

193   with the sample it was rinsed with 0.05M ammonium acetate buffer for 2 minutes. The columns were

194   eluted with ~1.5ml 1M HNO$_3$ and the eluent was analyzed in triplicate on the SF-ICP-MS run in

195   medium-resolution mode. External precision for obtained [Zn^{2+}] was 5.19%, and we obtained an average

196   [Zn^{2+}] of 0.036 mg/L or 554 ± 21 nM (Table 2), which is over 100 times higher than natural deep

197   seawater concentrations, based on global deep water Si:Zn relationship (Marchitto et al., 2000). This

198   concentration of Zn/Ca$_{SW}$ (average of 66 ± 3 µmol/mol) is approximately 15x higher than average

199   surface seawater (4.4 µmol/mol; Boyle, 1981; Marchitto et al., 2000). Still, this concentration is not

200   considered to be toxic to foraminifera (Nardelli et al., 2013).

201

202   **2.4.3 Elemental concentrations in foraminiferal calcite**

203   After termination of the experiment, foraminiferal shells were cleaned by adding pH buffered sodium

204   hypochloride (15% NaOCl) to microvials with individual foraminifera to remove organic material. After

205   incubation for 20 minutes, samples were rinsed three times with ultrapure water, then dried in a laminar

206   flow cabinet and positioned on a stub. Element concentrations of individual chambers were analyzed by

207   Laser Ablation-ICP-MS (Reichart et al., 2003). To determine foraminiferal Zn/Ca, the laser system

208   (NWR193UC, New Wave Research) at the Royal NIOZ was equipped with a 2-volume cell (New Wave

209   Research), characterized by wash-out time of 1.8 seconds (1% level) and hence allowing to detect

210   variability of obtained Zn/Ca within chamber walls. Single chambers were ablated in a helium

211   environment using a circular laser spot with a diameter of 80 µm. All foraminiferal samples were ablated

212   with an energy density of 1±0.1 J/cm^2 with a repetition rate of 5 Hz. The aerosol was transported with a

213   helium/argon flow into the quadrupole ICP-MS (iCAP-Q, Thermo Scientific). Monitored masses

214   included ^{23}Na, ^{24}Mg, ^{25}Mg, ^{26}Mg, ^{27}Al, ^{43}Ca, ^{44}Ca, ^{55}Mn, ^{66}Zn, and ^{88}Sr. ^{66}Zn is free of interferences when

215   measuring calcium carbonate and SRM NIST glass standards, contrary to ^{67}Zn and ^{68}Zn (Jochum et al.,

216   2012). Zn and Ca intensities over time were integrated with Thermo Qtegra software, avoiding potential

217   contamination or diagenesis of the outer or inner layer of calcite by monitoring the Al- and Mn-signals,

218   resulting in determined Zn/Ca of the experimental calcite only (Fig. 2). Due to the low Zn incorporation

219   in pre-experimental (field) chambers, we could also distinguish and exclude older chambers by absence

220   of a detectable Zn profile. Calibration was performed against SRM NIST612 glass standard, at an energy

221   density of 5±0.1 J/cm^2, with ^{43}Ca as an internal standard. Using a different energy density between

222   foraminiferal calcite and glass standard has been shown to not affect the analyses (Dueñas-Bohórquez

223   et al., 2009). NIST612 is a suitable calibration standard when measuring Zn/Ca, since Zn concentration

224   of this standard is relatively close (39.1 ppm) to that in the foraminiferal calcite from the experiments

225   used here. To increase precision and accuracy, we applied a linear drift correction over the series of

226   measurements. Relative precision of the analyses (relative standard deviation (RSD) of all NIST612) is

227   3% for ^{25}Mg, 3% for ^{88}Sr and 2% for ^{66}Zn. In total, 162 chambers were analyzed for eight experimental

228   conditions, resulting in 10-26 single chamber measurements per condition.

229   We calculated the standard deviation (SD), RSD and standard error (SD/√n; SE) per treatment. The

230   partitioning coefficient of an element (E) between seawater and foraminiferal calcite is expressed as D_E

231   $= (E/Ca_{CALCITE})/(E/Ca_{SW})$. Partition coefficients (D_{Mg}, D_{Sr} and D_{Zn}) and element versus calcium ratio

232   (Zn/Ca$_{CALCITE}$) were statistically compared using a two-sided T-test with 95% confidence levels. This

233   also allows for the calculation of 95% confidence intervals over the average per treatment. F-tests were

234   used to compare the variance in Zn/Ca values of the pH Stable and Acidification treatment (α=0.05).

235 **3. Results**

236

237 **3.1 Calcitic element concentrations**

238 Average elemental ratios in foraminiferal calcite of newly formed chambers (summarized in Table 3)

239 from all treatments were 2.10±0.38 mmol/mol for Mg/Ca, 1.36±0.13 mmol/mol for Sr/Ca and ranges

240 from 45.3-130.7 μmol/mol for Zn/Ca. A summary of the obtained $Zn/Ca_{CALCITE}$, $Mg/Ca_{CALCITE}$ and

241 $Sr/Ca_{CALCITE}$ values are shown in Table 4 and Fig. 3. The relative standard deviations (RSD) of the

242 different elements varies between 15.3-22.2% (Mg/Ca), 6.8-11.9% (Sr/Ca) and 11.5-28% (Zn/Ca). For

243 $Mg/Ca_{CALCITE}$ (ranging from 1.71-2.48 mmol/mol) there was no siginifcant relation with any of the

244 seawater carbonate parameters (pH, $[CO_3^{2-}]$, TA and DIC). $Sr/Ca_{CALCITE}$ of *A. tepida* varies between

245 1.31-1.40 mmol/mol over both treatments. The partion coefficient D_{Sr} significantly changes over a range

246 of either $[CO_3^{2-}]$, TA and DIC (Fig. 4), while there is no significant relation with pH (p>0.25).

247

248 3.2 Zn/Ca variability

249 The precision of Zn/Ca for the calibration standard (2%) was much smaller than the observed variability

250 between measurements on chambers from the same treatment, which show a relative standard deviation

251 of 11.5-28.0%. Foraminiferal Zn/Ca values from the variable pH treatments vary between 75.1 and

252 121.2 μmol/mol, and Zn/Ca of foraminifera cultivated at stable pH lies between 45.3 and 130.7

253 μmol/mol (Fig. 5). When plotting Zn incorporation as a function of either TA or DIC (Fig. 5a, b),

254 $Zn/Ca_{CALCITE}$ decreases with both increasing TA and DIC in the pH stable treatment, resulting in an

255 overall correlation with both these parameters (p<0.05). However, average $Zn/Ca_{CALCITE}$ varies strongly

256 within the Acidification experiment, over a narrow range in TA and DIC. When plotting $Zn/Ca_{CALCITE}$

257 versus pH (Fig. 5c), we observe a large range in Zn/Ca values (45.3-130.7 μmol/mol) when pH remains

258 relatively stable (7.9-8.08). When comparing the variance in Zn/Ca values for the pH Stable group with

259 the Acidification group using a F-test, we observe that the variance of the Acidification group is

260 significantly smaller than the pH Stable group (left-sided F-test, α=0.05). This implies that the observed

261   variation in Zn incorporation is not controlled by pH, but rather by another parameter of the inorganic

262   carbon system that somewhat covaries with pH in the Acidification treatment (e.g. $[CO_3^{2-}]$). The best

263   overall fit is shown when Zn incorporation is plotted as a function of $[CO_3^{2-}]$ (Fig. 5d; $p<0.001$). We

264   obtain a highly significant relation ($p<0.0005$) when D_{Zn} ranges from 1.9 to 0.7 and $[CO_3^{2-}]$ changes

265   from 61.9 to 1081.7 µmol/kg (Fig. 6).

266 **4. Discussion**

267 **4.1 Chamber addition rates**

268 When comparing the average chamber addition of the foraminifera in this study (Table 3) with a

269 foraminiferal culture study with enriched heavy metals (Munsel et al., 2010), we find almost 10-fold

270 higher chamber addition rates per week (0.23 versus 0.03-0.04 chambers per week in the 10- and 20-

271 fold enrichment treatment). This could be due to additional stress in the study by Munsel et al. (2010),

272 by increasing the concentration of three heavy metals (Cu, Ni and Mn) instead of one, or due to the

273 introduction of fluorescent label calcein. Long-term exposure to calcein has an adverse effect on benthic

274 foraminifera, even when using low concentration of 5mg/L (Kurtarkar et al., 2015), which may explain

275 the overall lower new chamber yield in the culture study of Munsel et al. (2010).

276

277 **4.2 Element incorporation as a function of seawater carbonate chemistry**

278 Culturing foraminifera under a range of inorganic carbonate conditions shows that incorporation of Zn

279 in shell calcite correlated particularly to $[CO_3^{2-}]$ (Fig. 5d), since the range in foraminiferal Zn/Ca is

280 relatively large when either of the other components (i.e. pH, TA and DIC) are stable or vary over a

281 limited range. Although co-variation is observed with pH, TA and DIC of the culture media (Fig. 6a-c)

282 this seems an artefact from the inherent coupling of these parameters in the seawater carbonate system.

283 This suggests that Zn/Ca$_{CALCITE}$ provides a robust proxy for carbonate ion concentration. No impact of

284 seawater pH, TA and/or DIC is necessary to explain the observed trends. In contrast to Zn/Ca$_{CALCITE}$,

285 foraminiferal Sr/Ca decreases when $[CO_3^{2-}]$, TA and/or DIC increases (Fig. 4 and Table 4). Sr

286 incorporation in foraminiferal calcite has been suggested to change with pH (Lea et al., 1999), and later-

287 on has been proposed to react to changes in $[CO_3^{2-}]$ (Dissard et al., 2010). We show that D_{Sr} is not

288 significantly impacted by pH (Fig. 4), but by either $[CO_3^{2-}]$, DIC or TA. Foraminiferal Mg/Ca (2.10±0.38

289 mmol/mol) are in good agreement with the expected Mg/Ca (2.17 mmol/mol) based on existing

290 temperature calibrations for this species (Toyofuku et al., 2011). Moreover, Mg/Ca does not vary

291 systematically with seawater carbonate chemistry, confirming results from earlier studies (e.g. Dueñas-

292 Bohórquez et al., 2011), but are in contrast with others (Kısakürek et al., 2008; Evans et al., 2016).

293

4.3 Intra- and inter-specimen Zn/Ca variability

In our study we observed appreciable intra- and/or inter-specimen variability in Zn/Ca$_{CALCITE}$. The relative standard deviation (RSD) of measured Zn/Ca$_{CALCITE}$ between specimens ranged from 11.5 to 28.0%, depending on the treatment (Table 4). This variability is considerably higher than the external precision (RSD = 2%), based on multiple analyses of the calibration standard (NIST612). One of the causes for this relative high variability in the Zn/Ca of foraminifera might be laboratory contamination. Zn/Ca$_{CALCITE}$ has been proven challenging to measure (Bruland et al., 1978), due to the combination of generally low concentrations in calcium carbonate and potential contamination when handling samples in the laboratory. Therefore, to optimize our Zn/Ca$_{CALCITE}$ measurements, we did not apply an acid leach during cleaning of the foraminiferal samples since this may increase Zn/Ca$_{CALCITE}$ values (Marr et al., 2013). We also increased the initial Zn concentrations in the culture water and cultured foraminifera without natural sediment to avoid contamination by presence of Zn-containing clay particles. Therefore, contamination is an unlikely cause for the observed inter- and/or intraspecimen variability in Zn/Ca. Zn can also be introduced in the culture vessels by the addition of food, since the growth media for algae contains measurable concentrations of Zn. However, since we separated this culture media from the *Dunaliella* cells by centrifuging and freeze-drying, we assume this amount of additional Zn is either absent or negligible.

Another source for the variability in Zn/Ca$_{CALCITE}$ within treatments might be the stability of the experimental set-up over time. Due to the slope of the relationship between D$_{Zn}$ and [CO$_3^{2-}$] (Fig. 6), small deviations of pCO$_2$ during the culture experiment may have caused variability in [CO$_3^{2-}$] and thereby, minor variation in intra- and/or inter-variability of Zn/Ca$_{CALCITE}$. Relative stability of TA (RSD <8.6%) and [DIC] (RSD <4.4 %) during the course of the experiments (Table 1), however, suggest that variability in [CO$_3^{2-}$] was minor. Nevertheless, changes in seawater carbonate chemistry on time scales shorter than that required to build a shell and/or single chamber could result in a heterogeneous Zn distribution between foraminiferal shell chambers. Even though short-term variability might not have been detected due to the frequency of the sampling, it unlikely accounts for the range in measured Zn/Ca$_{CALCITE}$, as this would have averaged out in the time needed for a foraminifera to add chambers.

321   Variability in Zn/Ca, like other proxies based on foraminiferal carbonate, may be explained by

322   differences in e.g. ontogeny, growth rates and biological activity (Zeebe et al., 2008; Raitzsch et al.,

323   2010; Wit et al., 2012), which all might affect element fractionation during biomineralization (Erez,

324   2003; De Nooijer et al., 2014b). In a number of studies (Erez, 2003; Eggins et al., 2004; Sadekov et al.,

325   2005; Kunioka et al., 2006; Hathorne et al., 2009; De Nooijer et al., 2014a; Branson et al., 2015) it has

326   been shown that other elements like Mg, Ba and B can be heterogeneously distributed within chamber.

327   In our study, besides a relatively high variability in Zn/Ca$_{CALCITE,}$ we also observed similar high RSD

328   values for Mg/Ca$_{CALCITE}$ (15.3-22.2%), which might indicate that Zn is also incorporated

329   heterogeneously. This high variability, caused by heterogeneity in Zn distribution within a foraminifera

330   might limit the precision of this potential proxy. However, it has been shown for foraminiferal

331   Mg/Ca$_{CALCITE}$ that the trend between the margin of error and sample size ($1/\sqrt{n}$; Sadekov et al., 2008;

332   De Nooijer et al., 2014a) is in line with a more or less random distribution. Hence oversampling and

333   statistical treatment of the data should cancel out such effects. Like Mg/Ca$_{CALCITE}$, the uncertainty in

334   estimated average Zn/Ca$_{CALCITE}$ would then also decrease exponentially with increasing sample size.

335   Such an averaging corresponds with the within chamber-wall variability in Zn being primarily related

336   to a lamellar mode of calcification (Reiss, 1960). The minimum number of specimens required for an

337   accurate estimate of average population Zn/Ca depends on the constraints needed for the $[CO_3^{2-}]$

338   concentration, following similar models as previously published for Mg/Ca and temperature (Wit et al.,

339   2012).

340

341   **4.4. Zinc is incorporated as Zn^{2+}**

342   To explore the mechanisms underlying incorporation of Zn in foraminiferal calcite, activities of different

343   species of Zn were modeled as a function of changing $[CO_3^{2-}]$ using the software package PHREEQC

344   (Parkhurst and Appelo, 1999) and the standard PHREEQC llnl database. For all experimental treatments,

345   free Zn (Zn^{2+}) increases with increasing carbonate ion concentration. Simultaneously, activity of

346   aqueous Zn-carbonate complexes (ZnCO$_3^0$ and ZnHCO$_3^+$) decreased or remained similar over the range

347   of $[CO_3^{2-}]$ studied here. These modeled activities were plotted against the average Zn/Ca$_{CALCITE}$

348   corresponding the $[CO_3^{2-}]$ from our treatments (Fig. 7). The incorporation of Zn in foraminiferal shell

349   calcite increased with Zn^{2+} activity for both the pH stable and the Acidification treatments. When

350   running the model with normal (15 times lower) [Zn], we find similar profiles for the activity of different

351   Zn species. This suggests that the incorporation of Zn is mainly determined by the (bio-) availability of

352   Zn as free Zn^{2+} ion, which is in turn affected by changes in $[CO_3^{2-}]$, and are not impacted by the total

353   [Zn]. However, the slope of the relationship between Zn/Ca $_{CALCITE}$ and Zn^{2+} activity is slightly different

354   for the two seawater manipulations, hinting at an additional control on the incorporation of Zn. This

355   might indicate either that $[CO_3^{2-}]$ influences other mechanisms that control Zn incorporation, e.g. growth

356   rates (both chamber addition and calcification rate), metabolism, ontogeny, or that a second (carbon)

357   parameter secondarily affects Zn-incorporation. The second possibility is less likely, due to the observed

358   strong correlation between $[CO_3^{2-}]$ and D_{Zn} ($p<0.0005$). Nevertheless, we cannot exclude TA or DIC as

359   a potential secondary, minor control on Zn in foraminiferal shell calcite. Of all parameters investigated

360   here, pH might be the least likely to have an effect on Zn-incorporation, since the observed change in

361   incorporated Zn is largest when pH remains stable.

362

363   **4.5 Inorganic precipitation versus biomineralization**

364   Even though inorganic calcite precipitation is not directly comparable to foraminiferal calcification

365   (Elderfield et al., 1996), comparing results of these two types of experiments may increase our

366   understanding of some of the controls on element incorporation during biomineralization (De Nooijer

367   et al., 2014b). Results from inorganic adsorption experiments (Zachara et al., 1991) show that the

368   relation between calcite Zn/Ca and $[CO_3^{2-}]$ is similar to that found here for the benthic foraminifer

369   *Ammonia tepida*. Inorganically precipitated calcite crystal surfaces have a higher affinity for Zn as pH

370   of the growth medium increases. In a study by Zachara et al. (1991) pH of different treatments was

371   changed by manipulating DIC (although not explicitly stated in the paper), which implies that e.g.

372    $NaHCO_3$ was added to achieve the desired solution composition and pH, resulting a similar anti-

373   correlation of pH and $[CO_3^{2-}]$ created in the experiments presented here. However, in the experiments

374   of Zachara et al. (1991), where the solution was in equilibrium with calcite, the calcite surface

375   characteristics will vary with pH. With progressively higher pH, the surface becomes more negatively

376   charged, enhancing the adsorption of positively charged ions such as Zn^{2+} (Wolthers et al., 2008). In our

377 culturing study, with solutions that are supersaturated with respect to calcite, there is an opposite trend:

378 a decrease in Zn association with pH (Fig. 5c). Clearly, the local control of the foraminifera on the

379 calcifying fluid (De Nooijer et al., 2009; Glas et al., 2012), combined with the impact of free Zn^{2+} activity

380 overrule any potential surface charge effects on Zn uptake. During calcification in perforate species,

381 Ca^{2+} is actively taken up in the cytoplasm via transmembrane transporters, and is probably not stored in

382 intravesuclar reservoirs (De Nooijer et al., 2014b). Our results suggest that uptake of Zn^{2+} follows the

383 same pathways as Ca^{2+}, with more Zn^{2+} being incorporated with increasing availability of free Zn^{2+}. In

384 our culture study, foraminiferal D_{Zn} varies between 1.9 and 0.7, while inorganically precipitated calcite

385 has a D_{Zn} of approximately 6 at similar temperature and pressure, but higher seawater [Zn] (2120 nM;

386 Crocket and Winchester, 1966). This suggests that foraminifera incorporate less Zn than expected from

387 an a-biological perspective. Such an apparent discrimination against Zn is in line with uptake over a

388 membrane as suggested above.

389

390 **4.6 The effect of calcite saturation state on D_{Zn}**

391 Marchitto et al. (2005) found a positive relation between calcite saturation state, expressed as ΔCO_3^{2-}

392 ($[CO_3^{2-}]_{in\ situ}$ - $[CO_3^{2-}]_{saturation}$) and D_{Zn}. A similar effect has been reported for other elements, like D_{Cd} and

393 D_{Ba} by McCorkle et al. (1995), who showed reduced incorporation of trace metals in undersaturated

394 waters. Apparently, the mechanics behind the relation between $[CO_3^{2-}]$ and Zn incorporation found in

395 our study and the studies of Marchitto et al. (2000, 2002 and 2005) stem from two different controls.

396 These last studies analyzed foraminiferal Zn in deep-sea foraminifera of core-tops in near-

397 undersaturated seawater and argues Rayleigh fractionation causes the internal calcification reservoir to

398 be flushed rapidly, resulting in lower D_{Zn} due to lower calcite saturation state. In our case, the culture

399 media were always well above calcite saturation state (lowest $\Omega = 1.49$; Table 1). This might indicate

400 that the influence of near-undersaturation with respect to calcite does not effect our data, in which the

401 incorporation of Zn is mainly governed by the amount of bioavailability of Zn^{2+}.

402 There is a distinct offset observed between estimates of *Cibicidoides wuellerstorfi* (D_{Zn}=9; Marchitto et

403 al., 2000), *Cibicidoides pachyderma* (D_{Zn}=22; Bryan and Marchitto, 2010), the miliolid

404 *Pseudotriloculina rotunda* (D_{Zn}=0.2-4.0; Nardelli et al., 2016) and our culture calibration for *Ammonia*

405   *tepida* (D_{Zn}=1.9-0.7). This offset could be caused by the different seawater [Zn], due to difference in

406   fractionation in natural conditions. Nardelli et al. (2016) show that lower seawater [Zn] results in higher

407   D_{Zn}, which could explain why Zn partitioning is higher in the *Cibicidoides* species, which probably

408   calcified at lower (deep sea) seawater [Zn]. Furthermore, observed differences might stem from species-

409   specific vital effects due to calcification strategy, contamination, study type (culturing versus field-

410   calibration) and/or accuracy in determination of $Zn/Ca_{SEAWATER}$, but might also reflect an additional

411   environmental control on Zn incorporation. Temperature, for instance, influences crystal growth rates

412   (Morse et al., 2007), which consequently might have an effect on the sorption and subsequent

413   incorporation of metals on the crystal structure. Also other environmental factors, e.g. salinity, not

414   constrained here, might affect these partitioning coefficients.

415

416   **4.7 Paleoceanographic implications**

417   The relationship between [CO_3^{2-}] and foraminiferal Zn/Ca could be applied for reconstructing two types

418   of parameters. Firstly, our results show that foraminiferal Zn/Ca might indeed be used as a proxy for

419   [CO_3^{2-}], and furthermore, that it also is not biased by changes in pH. However, when aiming to

420   reconstruct [CO_3^{2-}] using $Zn/Ca_{CALCITE}$ one must take into account the difference in behavior of D_{Zn}

421   versus [CO_3^{2-}] due to undersaturation effect discussed in section 4.6. Furthermore, as Zn in ocean water

422   follows a nutrient-like behavior, an independent estimate of past seawater [Zn] is necessary. For

423   instance, Bryan and Marchitto (2010) show that in thermocline waters, $Zn/Ca_{CALCITE}$ of *Cibicidoides*

424   *pachyderma* increases with increasing [CO_3^{2-}], however, [Zn] is not constrained in these samples. This

425   would allow for unravelling of past seawater composition and reconstruction of past nutrient profiles.

426   For instance, if U/Ca (Keul et al, 2013b) and Cd/Ca (Marchitto et al., 2002) are primarily controlled by

427   changes in [CO_3^{2-}] and the element to calcium ratio (E/Ca) of the seawater, simultaneous measurement

428   of these elements on a single foraminifera by LA-ICP-MS would results in several independent

429   reconstructions of [CO_3^{2-}]. Differences between these reconstructions reflect changes in the

430   concentrations of these elements relative to each other. When the errors of the different $E/Ca_{CALCITE}$

431   measurements are sufficiently low, it might be possible to reconstruct the concentrations of these

432   elements in seawater. Future research must focus on confirming these proxies (e.g. Zn/Ca, Cd/Ca and

433   U/Ca) as being mainly controlled by $[CO_3^{2-}]$, and decreasing the error in their relationship to $[CO_3^{2-}]$.

434   When past seawater Zn concentration is known, the global deep water Zn:Si relationship $[Zn] =$

435    $0.052[Si] + 0.79$ with $[Zn]$ in nmol/kg and $[Si]$ in µmol/kg (Marchitto et al., 2000) can be used to

436   estimate past $[Si]$ (profiles) of the ocean. Moreover, since the ratio between $[Zn]$ and $[Cd]$ varies between

437   water masses, relative changes in these elements may reflect changes in oceanic circulation, as has been

438   shown by Marchitto et al. (2002). So reconstructing seawater E/Ca by using an independent $[CO_3^{2-}]$

439   proxy, in theory, one will be able to reconstruct past seawater element composition and even ocean

440   circulation.

441   Secondly, when past seawater Zn/Ca is constrained, the relationship between carbonate ion

442   concentration and Zn incorporation in *Ammonia tepida* can be used to reconstruct a change in $[CO_3^{2-}]$

443   from e.g. 200 to 300 µmol/kg, which would result in an increase in foraminiferal Zn/Ca of 9.5% (Fig.

444   5). In combination with a proxy for other inorganic carbonate system parameters (e.g. $\delta^{11}B$ from

445   foraminiferal calcite for seawater pH; Hönisch et al., 2009; Sanyal et al., 1996), Zn/Ca can enable

446   reconstruction of the complete carbon system and thereby, past atmospheric CO_2. However, currently,

447   the sensitivity of the relationship between $Zn/Ca_{CALCITE}$ and $[CO_3^{2-}]$, in combination with the relative

448   large variability in $Zn/Ca_{CALCITE}$, is too large to reconstruct meaningful changes in $[CO_3^{2-}]$ smaller than

449   interglacial-glacial cycles. Including multiple elements with a similar type of mixed nutrient/carbonate

450   system behavior could help to improve such reconstructions.

5. Conclusions

We decoupled pH and carbonate ion concentration in a controlled growth experiment, culturing the benthic foraminifer *Ammonia tepida*. Impact of individual inorganic carbonate parameters on Zn incorporation in the shell calcite was determined using laser ablation ICP-MS measurements of individual shell chambers. This showed that foraminiferal Zn/Ca decreases with increasing $[CO_3^{2-}]$, with only minor impacts of pH, DIC and TA. Modelling Zn speciation in seawater showed that the amount of bioavailable zinc in seawater decreases with increasing $[CO_3^{2-}]$, in line with the observed Zn/Ca values in the foraminiferal shells. This results confirm an earlier field calibration of Zn/Ca values to changes in $[CO_3^{2-}]$ (Marchitto et al., 2000), and shows Zn incorporation to be independent from pH. Therefore, we argue that foraminifera Zn/Ca values, combined with an independent other $[CO_3^{2-}]$ proxy, potentially allow reconstructing past nutrient profiles. Conversely, combining foraminiferal Zn/Ca with an other independent carbonate system proxy (for e.g. pH), enables reconstruction of the complete carbon system and thereby, past atmospheric CO_2.

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Treatments		Measured					Calculated CO2SYS			
		pH	TA µmol/kg		DIC µmol/kg		atm CO_2 ppm	pH (total scale)	$[CO_3^{2-}]$ µmol/kg	$\Omega_{CALCITE}$
			Avg±SD	RSD	Avg±SD	RSD				
Stock		8.21±0.02	2397±4	2.3	2230±11	1.0	300.1	8.15	122.6	2.94
Acidification	180	8.28±0.03	2899±19	6.5	2278±63	2.8	193.6	8.37	457.9	11.02
	760	8.01±0.01	2518±17	6.6	2295±43	1.9	692.2	7.88	172.0	4.14
	1400	7.84±0.02	2345±11	4.8	2252±51	2.3	1332.3	7.60	91.1	2.19
	2000	7.61±0.04	2240±19	8.6	2203±77	3.5	1896.1	7.44	61.9	1.49
pH stable	180	7.94±0.04	994±8	8.1	835±37	4.4	196.3	7.98	77.6	1.87
	760	7.95±0.02	3886±13	3.2	3461±55	1.6	728.9	8.02	352.1	8.48
	1400	8.01±0.02	7341±28	3.8	6600±62	0.9	1358.8	8.03	684.2	16.47
	2000	8.08±0.03	11025±26	2.4	9898±97	1.0	1910.4	8.06	1081.7	26.04

Table 1. Average (Avg) carbon parameters (pH, n=12; TA= Total alkalinity; n=6 DIC=Dissolved Inorganic Carbon, n=6) with (relative) standard deviation of the culture water per treatment. CO2SYS was used to calculate carbonate ion concentration ($[CO_3^{2-}]$) and calcite saturation state ($\Omega_{CALCITE}$) and recalculate pH and atmospheric CO_2 from measured TA and DIC.

Treatment		$[Zn^{2+}]_{sw}$ nM	$[Mg^{2+}]_{sw}$ mM	$[Sr^{2+}]_{sw}$ µM	$[Ca^{2+}]_{sw}$ mM	Zn/Ca$_{sw}$ µmol/mol	Mg/Ca$_{sw}$ mol/mol	Sr/Ca$_{sw}$ mmol/mol
Acidification	180	563.53 ±12.82	44.54 ±1.78	53.56 ±3.28	8.57 ±0.08	**65.79 ±2.15**	**5.20 ±0.26**	**6.25 ±0.45**
	760	517.59 ±23.65	45.70 ±3.20	49.47 ±1.35	8.51 ±0.44	**60.80 ±5.89**	**5.38 ±0.65**	**5.82 ±0.46**
	1400	570.72 ±31.25	46.87 ±1.41	53.46 ±1.08	8.68 ±0.78	**65.73 ±9.51**	**5.41 ±0.65**	**6.17 ±0.68**
	2000	582.20 ±67.03	47.28 ±2.36	51.41 ±1.15	8.41 ±0.0.48	**69.24 ±11.92**	**5.63 ±0.60**	**6.13 ±0.49**
pH stable	180	537.49 ±5.74	44.62 ±1.34	50.07 ±3.05	7.95 ±0.72	**67.60 ±6.81**	**5.62 ±0.68**	**6.31 ±0.95**
	760	560.96 ±6.42	46.07 ±0.46	54.98 ±2.97	8.52 ±0.62	**65.84 ±5.50**	**5.42 ±0.45**	**6.47 ±0.82**
	1400	542.4 ±22.63	49.79 ±2.49	47.99 ±3.88	8.56 ±0.35	**63.43 ±5.04**	**5.84 ±0.53**	**5.62 ±0.68**
	2000	553.32 ±13.44	48.85 ±0.49	36.22 ±1.58	8.13 ±0.44	**68.10 ±4.51**	**6.02 ±0.39**	**4.47 ±0.44**

Table 2. Element concentrations and element-calcium ratios (bold) of the culture water per treatment ± (propagating) standard deviation.

Treatment		Total n of foraminifera	Foraminifera that formed new chambers		Total n of added chambers	Avg. chamber addition	
			n	(%)		42 days (Avg±SD)	Per week
Acidification	180	12	12	100	22	1.8±0.7	0.26±0.10
	760	15	15	100	22	1.5±0.9	0.21±0.13
	1400	11	8	73	18	1.6±0.7	0.23±0.10
	2000	12	12	100	16	1.3±1.0	0.19±0.15
pH stable	180	11	10	91	18	1.6±0.4	0.23±0.06
	760	11	10	91	16	1.5±0.5	0.21±0.07
	1400	10	9	90	17	1.7±0.7	0.24±0.10
	2000	15	13	87	29	1.9±0.7	0.28±0.10

Table 3: Number of new chambers added per treatment. Average chamber addition (per week) based on linear growth.

Treatment		N	Zn/Ca$_{CALCITE}$ μmol/mol				D_{Zn}	Mg/Ca$_{CALCITE}$ mmol/mol				D_{Mg} *10^{-3}	Sr/Ca$_{CALCITE}$ mmol/mol				D_{Sr}
			Avg	SD	RSD	SE		Avg	SD	RSD	SE		Avg	SD	RSD	SE	
Acidification	180	22	75.1	21.0	28.0	4.5	**1.1**	1.71	0.38	22.2	0.12	**0.33**	**1.38**	0.14	10.5	0.05	**0.22**
	760	28	93.4	12.9	13.8	2.4	**1.5**	2.10	0.38	18.1	0.08	**0.39**	**1.35**	0.12	9.2	0.03	**0.23**
	1400	18	108.2	12.5	11.5	2.9	**1.6**	1.84	0.37	20.1	0.09	**0.34**	**1.31**	0.12	8.8	0.04	**0.21**
	2000	23	121.2	15.8	13.0	3.3	**1.8**	2.46	0.38	15.4	0.08	**0.44**	**1.31**	0.11	8.7	0.03	**0.21**
pH stable	180	15	130.7	21.7	16.6	5.6	**1.9**	2.02	0.38	18.8	0.09	**0.36**	**1.36**	0.12	8.8	0.04	**0.22**
	760	15	81.0	19.4	24.0	5.0	**1.3**	2.48	0.38	15.3	0.10	**0.46**	**1.37**	0.09	6.8	0.04	**0.21**
	1400	15	57.5	13.6	23.6	3.5	**0.9**	2.05	0.39	19.0	0.10	**0.35**	**1.39**	0.17	11.9	0.04	**0.25**
	2000	26	45.3	9.1	20.0	1.8	**0.7**	2.15	0.35	16.3	0.07	**0.36**	**1.40**	0.13	9.6	0.03	**0.31**

Table 4. Overview of Zn/Ca, Mg/Ca, and Sr/Ca ratios in foraminiferal calcite (Avg=average; SD=standard deviation; RSD=relative standard deviation; SE=standard error) results from LA-ICP-MS, and partitioning coefficients D_E in bold.

Figure 1. Culture set-up: CO_2 premix connected to an air tight incubator containing two sets of culture samples and both artificial seawater treatments, 'pH stable' and 'Acidification'. The total set-up comprised four incubators, each coupled to one of four different CO_2 premixes.

Figure 2. Example of a typical LA-ICP-MS analysis of a single chamber. Gas blanks are deducted from the signal in the integrated period, to obtain elemental concentration of primary calcite.

Figure 3. Mg/Ca$_{CALCITE}$ (a; diamonds), Sr/Ca$_{CALCITE}$ (b; circles) and Zn/Ca$_{CALCITE}$ (c; squares) average±SD element to calcium ratios and LA-ICP-MS single measurements of both Acidification (white symbols and grey dots respectively) and pH Stable (red symbols and pink dots resp.) with related boxplots for all treatments (A-D).

Figure 4. D$_{Sr}$±SD of the pH stable (red) and Acidification (white) treatment. Top panel: D$_{Sr}$ versus a) Total Alkalinity (TA) and b) Dissolved Inorganic Carbon (DIC). Lower panel: D$_{Sr}$ versus c) pH and d) [CO_3^{2-}]. Grey dashed lines are linear regressions, R^2 and p-vales (95% confidence) are reported in the graph.

Figure 5. Zn/Ca$_{CALCITE}$ of the pH stable (red) and Acidification (white) treatment. Top panel: Zn/Ca$_{CALCITE}$ versus a) Total Alkalinity (TA) and b) Dissolved Inorganic Carbon (DIC). Lower panel: Zn/Ca$_{CALCITE}$ versus c) pH and d) [CO_3^{2-}]. Standard errors (solid line) and -deviations (dotted line), caused by intra and inter specimen variablity are plotted for every treatment. Grey dashed lines are linear regressions, R^2 and p-vales are noted in the graph.

Figure 6. Partitioning coefficient, $D_{Zn} \pm$ SD of individual ablations (grey circles) and the average per treatment (pH stable = red, Acidification = white). The relationship between D_{Zn} (Y) and $[CO_3^{2-}]$ (X) can be expressed as $Y = ae^{bx}$, where a= 1.86±0.38 and b= -0.001±0.0001 (solid line). Dashed lines indicate 95% confidence interval.

Figure 7. Zn/Ca in foraminiferal calcite at different activities of free Zn^{2+} (left panel) and Zn-carbonate complexes (right panel; triangles= $ZnCO_3^0$; diamonds= $ZnHCO_3^+$) corresponding with the $[CO_3^{2-}]$ of the different treatments and seawater manipulations (pH stable = red; Acidification = white)

Acidification   pH stable

CO$_2$
+
air

2000 ppm
1400 ppm
760 ppm
180 ppm

a

Zn/Ca $_{CALCITE}$ (µmol/mol)

R^2=0.76
p<0.05

[TA](µmol/kg)

b

□ Acidification
■ pH Stable

Zn/Ca $_{CALCITE}$ (µmol/mol)

R^2=0.73
p<0.05

[DIC](µmol/kg)

c

Zn/Ca $_{CALCITE}$ (µmol/mol)

R^2=0.35
p<0.10

pH (total scale)

d

Zn/Ca $_{CALCITE}$ (µmol/mol)

R^2=0.86
p<0.001

[CO$_3^{2-}$](µmol/kg)