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Original Article

Interpreting the role of pH on stable isotopes in large benthic foraminifera

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Large benthic foraminifera (LBF) are prolific producers of calcium carbonate sediments in shallow, tropical environments that are being influenced by ocean acidification (OA). Two LBF species, *Amphistegina gibbosa* (Order Rotaliida) with low-Mg calcite tests and *Archaias angulatus* (Order Miliolida) with high-Mg calcite tests, were studied to assess the effects of pH 7.6 on oxygen and carbon isotopic fractionation between test calcite and ambient seawater. The $\delta^{18}O$ and $\delta^{13}C$ values of terminal chambers and of whole adult tests of both species after 6 weeks were not significantly different between pH treatments of 8.0 and 7.6. However, tests of juveniles produced during the 6-week treatments showed significant differences between $\delta^{18}O$ and $\delta^{13}C$ values from control (pH 8.0) when compared with the treatment (pH 7.6) for both species. Although each individual's growth was photographed and measured, difficulty in distinguishing and manually extracting newly precipitated calcite from adult specimens likely confounded any differences in isotopic signals. However, juvenile specimens that resulted from asexual reproduction that occurred during the experiments did not contain old carbonate that could confound the new isotopic signals. These data reveal a potential bias in the design of OA experiments if only adults are used to investigate changes in test chemistries. Furthermore, the results reaffirm that different calcification mechanisms in these two foraminiferal orders control the fractionation of stable isotopes in the tests and will reflect decreasing pH in seawater somewhat differently.

Keywords: adults and juvenile specimens, large benthic foraminifera, ocean acidification, oxygen and carbon isotopes, pH.

Introduction

Carbonate chemistry of surface seawater is changing as it maintains equilibrium with increasing atmospheric carbon dioxide concentration [CO_{2atm}] (Doney *et al.*, 2009). Models suggest that when [CO_{2atm}] reaches 1300 ppm, average pH of global seawater could be as low as 7.6, with a calcite saturation state (Ω_c) of 2.0 (Pörtner *et al.*, 2014). These projected changes in both pH and Ω_c are collectively referred to as ocean acidification (OA; Caldeira and Wickett, 2003), and laboratory experiments suggest that OA will significantly influence calcifying marine organisms with potential impacts soon (Doney *et al.*, 2009). Meta-analysis of these biological responses, however, has shown great complexity with high variability between individual species (Kroeker *et al.*, 2010).

Large benthic foraminifera (LBF) are symbiont-bearing protists that are prolific calcium carbonate ($CaCO_3$) producers in both modern (~130 Mt year^{-1}; Langer, 2008) and past (BouDagher-Fadel, 2008) oceans. Recent studies have demonstrated a variety of significant biochemical and morphological responses to OA (e.g. Uthicke and Fabricius, 2012; Keul *et al.*, 2013; McIntyre-Wressnig *et al.*, 2013; Doo *et al.*, 2014 and references therein; Knorr *et al.*, 2015; Prazeres *et al.*, 2015). These variations, in part, are rooted in processes involved in test calcification, which varies between orders (Erez, 2003; Knorr,

2014). Less clear are the effects of the chemical changes associated with OA on the stable isotopic composition of LBF. This is problematic because stable isotopic composition of foraminiferal tests (expressed as $\delta^{18}O$ and $\delta^{13}C$ values) are used in many applications to constrain a broad spectrum of paleoenvironmental elements, which include characterizing paleotemperature (McCrea, 1950; Emiliani, 1955), calculating global ice-volume fluctuations (Shackleton and Opdyke, 1973), stratigraphic correlation (Imbrie *et al.*, 1984), interpreting organic C reservoirs (Shackleton, 1977), and reconstructing life processes (Grossman, 1987). In this study, we report on analyses of the tests of *Amphistegina gibbosa* d'Orbigny, 1839 (Order Rotaliida), subsequently abbreviated as *A. gibbosa*, and *Archaias angulatus* Fichtel and Moll, 1798 (Order Miliolida), subsequently abbreviated as *A. angulatus*, to assess the effects of low pH and higher CO_2 on stable isotopes as proxies of OA and the potential implications in paleoenvironmental reconstruction using these species. We chose culture experiments at pH 7.6 as a target value when $[CO_{2atm}]$ reaches 1300 ppm (Stocker *et al.*, 2013; Pörtner *et al.*, 2014).

Background on calcification mechanisms in *Amphistegina* and *Archaias*

Representatives of *Amphistegina* and *Archaias* were investigated to determine the effects of higher CO_2 and lower pH on light stable isotopes in their calcareous tests in part because members of their respective families are among the most prolific sediment producers (Hallock, 1981; Hallock *et al.*, 1986a; Langer *et al.*, 1997). In addition, these taxa have quite contrasting methods of calcification. *Amphistegina gibbosa* produces hyaline low-Mg calcite tests and calcifies through biological mediation (de Nooijer *et al.*, 2014), whereas *A. angulatus* produces porcelaneous, high-Mg calcite tests and calcifies using a less mediated, vacuolization process (de Nooijer *et al.*, 2009). Erez (2003) reported that calcification rates in *Amphistegina lobifera* increased essentially linearly between pH 7.0 and ~8.3, levelling off then declining at pH >9. In contrast, the larger miliolid *Amphisorus hemprichii* exhibited an exponential increase in calcification rates at pH between 7.0 and 9.5 (Erez, 2003).

Archaias angulatus (Figure 1) is a shallow-water (<30 m) species common throughout the western Atlantic and Caribbean. The test is planispiral and involute in early whorls, and is constructed of imperforate, porcelaneous calcite. Members of this species host the chlorophyte algal endosymbiont *Chlamydomonas hedleyi* (Lee *et al.*, 1974). The surface of an *A. angulatus* test is covered with pseudopores that enhance light transmission into the network of chamberlets. The test wall consists of two layers of high-Mg calcites; the outermost test layer consists of a continuous sheet of rhombohedral calcite tiles; the more voluminous, inner layer consists of randomly oriented calcite needles (Lynts and Pfister, 1967; Reid and Macintyre, 1998).

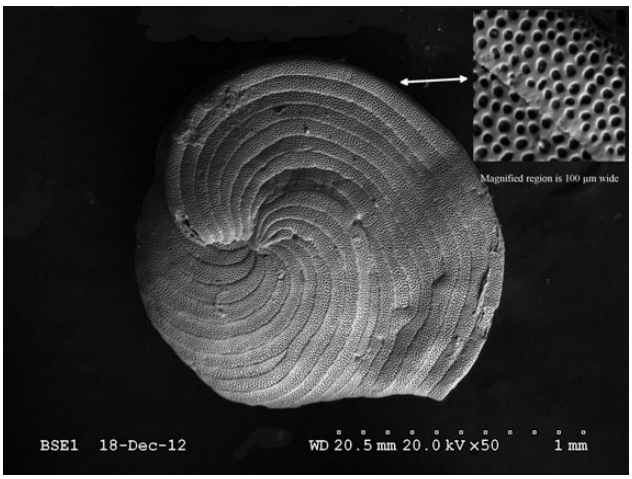

Figure 1. Scanning electron photomicrograph of *Archaias angulatus*. Scale bar = 1 mm.

Wetmore (1999) documented that chamber formation in *A. angulatus* occurs through the accretion of new chambers at the end of the test; overgrowth of older chambers may occur, but is an incidental consequence of growth geometry. When building a new chamber, the foraminifer first forms an organic membrane cyst with its reticulopodia, encapsulating and isolating space for new chamber construction. Meanwhile, a calcitic supersaturated solution is concentrated in seawater-filled vesicles within the cytoplasm, primarily the result of photosynthetic pH increases. This solution ultimately precipitates high-Mg calcite rods in the vesicles (Figure 2). The cytoplasm then transports the calcite rods to the cyst, where they are deposited. First, a single layer of calcite rhombohedra is placed along the extent of the new chamber; then, the remainder of the chamber wall is constructed, consisting of an unordered mass of calcite rods. Consistent with Erez's (2003) observation of pH influence of calcification rates in *Amphisorus*, Knorr *et al.* (2015) found a 50% reduction in growth rate of *A. angulatus* at pH 7.6.

Amphistegina gibbosa (Figure 3) is also a common western Atlantic and Caribbean shelf (<100 m) species. Its test is perforate, involute-trochospiral with a hyaline calcite composition. The test is divided into a series of interconnected chambers in which pennate diatom endosymbionts can be housed (Talge and Hallock, 2003). On the basis of the study of *A. lobifera*, Erez (2003) proposed that vacuoles of seawater, which originate by endocytosis, provide internal pools into which Ca^{2+} and CO_3^{2-} are concentrated by active transport that exchange Ca^{2+} ions for H^+ ions, thereby raising the pH within the vacuoles (Figure 4).

Amphistegina gibbosa grows using a biologically mediated process of the addition of overlapping new chambers at the end of the test (Hemleben *et al.*, 1986; Lowenstam and Weiner, 1989; Erez, 2003). The foraminifer extends its reticulopods around the test, forming a cytoplasmic substrate for calcification (Lowenstam and Weiner, 1989). The internal high pH pool, derived from both inorganic and metabolic carbon (ter Kuile *et al.*, 1989), supplies Ca^{2+} and CO_3^{2-} for calcification. Seawater CO_3^{2-} and HCO_3^- ions are the primary source for this internal carbon pool (ter Kuile and Erez, 1988) although the details of the ion acquisition mechanism of the carbon are ambiguous (de Nooijer *et al.*, 2014). The vacuoles containing concentrated Ca^{2+} and CO_3^{2-} are transported to the cytoplasmic substrate, where charged proteins draw the ions into place (Lowenstam and Weiner, 1989; Weiner and Dove, 2003). Consistent with Erez's (2003) observation of calcification rates in *A. lobifera* in response to pH, Knorr (2014) found a 12% reduction in growth rate of *A. gibbosa* at pH 7.6. McIntyre-Wressnig *et al.* (2013) reported no reduction in growth rates in *A. gibbosa* maintained for 6 weeks in pH treatments of ~7.4 and ~7.6 although they did find evidence of test dissolution in those specimens.

Material and methods

Sample collection

Live specimens of *A. angulatus* and *A. gibbosa* were harvested from the Keys Marine Laboratory Dolphin Cove (Layton, Florida Keys;

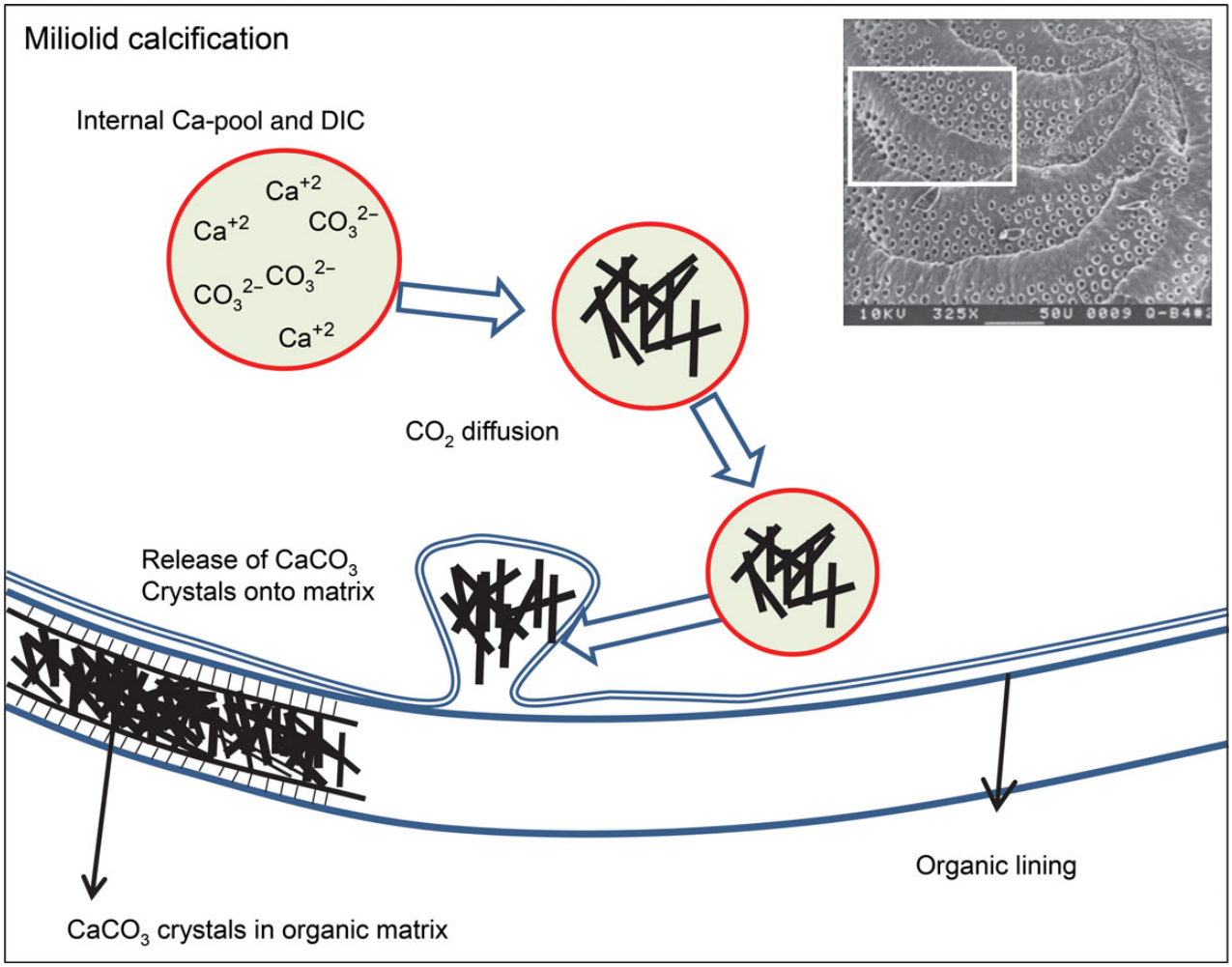

Figure 2. Depiction of calcification mechanism of *A. angulatus* modified after ter Kuile *et al.* (1989) and Cottey and Hallock (1988).

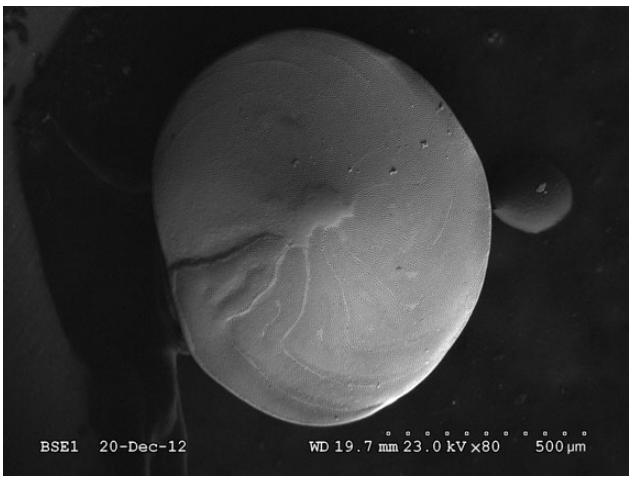

Figure 3. Scanning electron photomicrograph of *Amphistigina gibbosa*. Scale bar = 500 µm.

24.8268°N, 80.8144°W) and from nearby Tennessee Reef (24.775°N, 80.739°W), respectively. Both species were maintained in culture in St. Petersburg, FL, as described in Knorr *et al.* (2015). Briefly, groups of approximately 20 individuals from both species were photographed and placed into six plankton mesh bags, randomly consigned to six 38-L tanks maintained at target maintenance levels of 24°C, salinity of 34.9, and alkalinity of 2325 µmol kg^{-1}. The three control tanks were maintained at a headspace [$CO_{2(air)}$] average of 412 ± 3 ppm and pH_T of 8.0, whereas the three experimental tanks were maintained at a headspace [$CO_{2(air)}$] average of 700 ± 87 ppm before CO_2 injection and 1200 ± 265 ppm after injections with an average pH_T of 7.6.

On the basis of typical growth rates determined for *A. angulatus* (Hallock *et al.*, 1986a) and for *A. gibbosa* (Hallock *et al.*, 1986b; Talge and Hallock, 1995; Dettmering *et al.*, 1998), samples were grown in control and experimental tanks for 6 weeks, which allowed sufficient time to precipitate new $CaCO_3$ chambers as well as to asexually produce juvenile specimens. Mesh bags containing cohorts of *A. angulatus* and *A. gibbosa* were examined for adults from the original collection and for juvenile specimens that were produced during the experiment. These specimens were removed separately and prepared for isotope ratio mass spectroscopy. To compare the efficacy of selectively removing only the terminal chambers, which had formed under the control or experimental conditions, they were extracted from the tests of the adults (Knorr *et al.*, 2015) and combined to reach the 200 µg target mass needed for analysis. This 200 µg of adult specimens' terminal chambers was designated as the "last chamber" or "terminal chamber" sample. Whole tests from the juvenile specimens

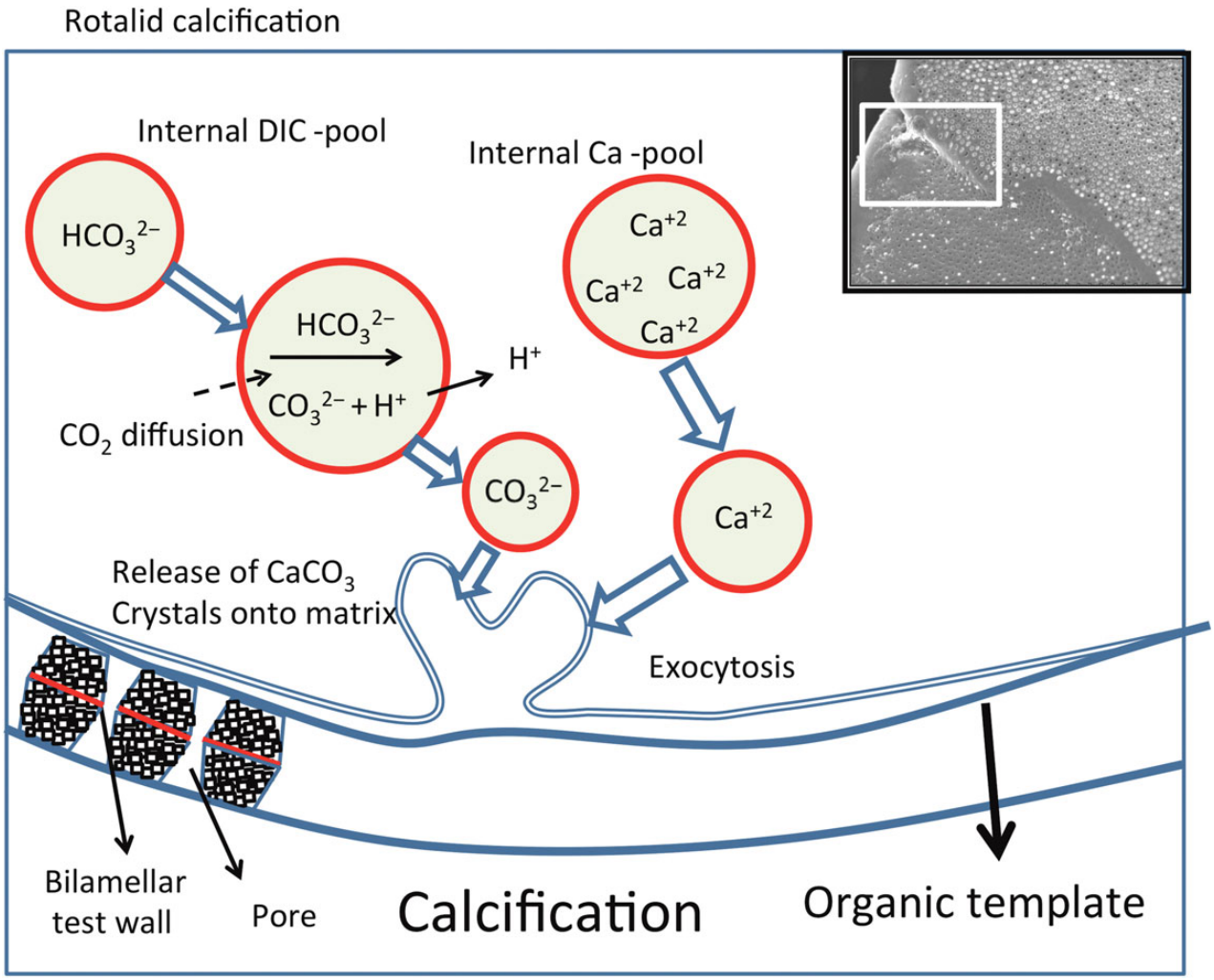

Figure 4. Depiction of calcification mechanism of *A. gibbosa* modified after ter Kuile *et al.* (1989) and de Nooijer *et al.* (2014).

were analysed since they had only experienced conditions within the tank in which they were produced and grew.

Measurement of carbonate chemistry

Target pH in the control (pH 8.0; $n = 3$) and experimental (pH 7.6; $n = 3$) tanks were achieved using a continuously monitored, automated pH-controlled CO_2 injection apparatus, which periodically injected $CO_{2(g)}$ into each tank headspace (Knorr, 2014). CO_2 was mixed with the tank water by a submersible aerator. The injection apparatus pH probes were calibrated 1–2 times weekly relative to concurrent total-scale pH spectrophotometric measurements. Spectrophotometric analyses of each tank pH_T and total alkalinity (A_T) were performed once or twice weekly and water chemistry was adjusted as necessary. The pH_T was measured with an Ocean Optics USB4000-UV-VIS fiber optic spectrophotometer and a thymol blue indicator following established methods (Clayton and Byrne, 1993; Dickson *et al.*, 2007). Total alkalinity (A_T) was measured using an open-cell titration with an Ocean Optics USB4000-UV-VIS fiber optic spectrophotometer and a bromocresol purple indicator (Yao and Byrne, 1998). The calibration of A_T was performed using Dickson certified reference materials (Dickson *et al.*, 2003). When necessary, reagent-grade $NaHCO_{3(s)}$ was dissolved in 50 ml of tank water and added to increase A_T values back to the target value. A second measurement of A_T was made 10 min after the end of the CO_2 injection cycle following the addition of $NaHCO_3$, at which time additional dilutions or additions were made as necessary. Previous trials determined that the addition of 100 μg of $NaHCO_3$ increased A_T by ~ 80 μmol kg_{sw}^{-1}. Kruskal–Wallis testing revealed no significant difference ($H_{23} = 7.31$, $p = 0.2$) between the A_T of the six tanks before and after $NaHCO_3$ addition.

Stable isotopic analyses

All stable isotopic analyses were completed at the University of South Florida's School of Geosciences Stable Isotope Laboratory on a Thermo Delta V isotope ratio mass spectrometer with the Gasbench II preparation system. Isotopic values for oxygen and carbon ($\delta^{18}O$ and $\delta^{13}C$ values) are reported on the delta scale ($\delta = R_{sample}/R_{standard} - 1$) in per mil notation (‰, where R is the ratio of heavy to light isotope for sample and standard). The standard reference scale used is Vienna Standard Mean Ocean Water (VSMOW) for $\delta^{18}O$ values for water and Vienna Pee Dee Belemnite (VPDB) for $\delta^{13}C$ and $\delta^{18}O$ values for calcite. For comparison of $\delta^{18}O$ of the calcite (on VPDB scale) and water (on VSMOW scale) under

equilibrium conditions, $\delta^{18}O$ values must be converted between VPDB and VSMOW scales. This conversion was done using the following equation:

$$\delta^{18}O_{VSMOW} = 1.03092\delta^{18}O_{VPDB} + 30.92‰. \quad (1)$$

Water samples from Dolphin Cove and tank experiments were collected for stable oxygen isotopic analysis of water and carbon isotopic analysis of dissolved inorganic carbon (DIC). Serum bottles (125 ml) were completely filled, fixed with 50 µl of saturated mercuric chloride, and sealed with greased Teflon crimp caps. Analyses of $\delta^{18}O$ of H_2O were completed by equilibrating 200 µl of sample with ~12 ml headspace of ~0.3% CO_2–He mixture in Labco Exetainers and allowing it to react for 24 h. Analyses of $\delta^{13}C$ of DIC were completed by injecting 1 ml aliquots of sample water into ~12 ml vials that were pre-flushed with He and pre-filled with 1 ml of 85% H_3PO_4 followed by equilibration for 24 h.

Juvenile and adult foraminiferal specimens from each cohort were sonicated in 25 µl of distilled, deionized water (DI) for 2 min, rinsed with 500 µl of DI water, dried at 60°C, and consolidated until the required analysis mass (~200 µg) was reached. Analyses of $\delta^{18}O$ and $\delta^{13}C$ of test calcite ($n = 30$) were completed by flushing 6 ml Labco Exetainers, containing ~100 µg of sample, with He gas, adding 2.5 ml of 103% phosphoric acid (H_3PO_4), and equilibrating for 24 h. In each analysis, the relevant isotopic composition ($\delta^{18}O$ or $\delta^{13}C$ values) of the headspace CO_2 was measured after equilibration was attained under controlled temperature conditions, and analyses normalized to internal reference standards.

Analytical precision (2σ) was <0.1‰ for $\delta^{18}O$ and $\delta^{13}C$ values of all water, DIC, and carbonate reference materials used as internal standards. Internal standards were anchored to the VPDB and VSMOW scales using international reference materials (NBS-19 and NBS-18 calcite, and VSMOW2, and SLAP2 water). Results were considered significant at $p < 0.05$; values are shown as (mean ± standard error).

Data analyses

Analysis of variance (ANOVA) with Tukey's pairwise comparisons was used to evaluate significant differences in the mean temperature, salinity, and alkalinity between experimental and control tanks. The general linear model (GLM) was used to evaluate the effect of pH on the oxygen and carbon isotopic ratios in the adult terminal chambers, adult whole tests, and juvenile whole tests of *A. angulatus* and *A. gibbosa*, in which the null hypothesis of primary interest was that there was no significant effect of pH on the isotopic values of tests. Using this approach, the factors (in nested order) were species, pH treatment, sample type (i.e. adult terminal chamber, adult whole test, and juvenile whole test), and replicate tanks, all of which were fixed. Data were analysed using the Minitab 17 statistical software and an α level of 0.05 was used in all tests.

Calculations

Unless influenced by vital effects, the $\delta^{18}O$ value of precipitated LBF calcite is highly dependent on that of local [HCO_3^-] and [CO_3^{2-}], precipitating as the weighted average of the two ions, which are in equilibrium with seawater (Zeebe, 1999). For precipitation of calcite from seawater, the $\delta^{18}O$ value of calcite in oxygen isotopic equilibrium with seawater can be predicted by mass balance:

$$\delta^{18}O_{calcite} = [CO_3^{2-}]\,(\varepsilon_{CO_3^{2-}-H_2O}) + [HCO_3^-](\varepsilon_{CO_3^{2-}-H_2O}). \quad (2)$$

This equation assumes enrichment factors between carbonate species (x) and seawater (ε_{x-H_2O}) of 34.3‰ for HCO_3^- and 18.4‰ for CO_3^{2-} [values calculated at a seawater temperature of 19°C, as in Zeebe (1999)]. HCO_3^- and CO_3^{2-} distribution at each pH are calculated using CO2calc (Robbins *et al.*, 2010), with inputs consisting of the mean temperature, salinity, and pH of the culture tanks, and carbonic acid dissociation constants (pK_1 and pK_2) from Lueker *et al.* (2000) and HSO_4^- dissociation constants from Dickson (1990) (Table 1).

Thus, at a seawater $\delta^{18}O$ value of 0, and pH of 7.6:

$$\delta^{18}O_{calcite} = 0.038\,(18.4) + 0.962(34.3) = 33.7‰_{\,VSMOW} = 2.7‰_{\,VPDB}, \quad (3)$$

and at pH 8.0:

$$\delta^{18}O_{calcite} = 0.097(18.4) + 0.903(34.3) = 32.8‰_{\,VSMOW} = 1.9‰_{\,VPDB}. \quad (4)$$

These calculations show that if the pH is reduced from 8.0 to 7.6 (at constant temperature), the $\delta^{18}O$ value will be enriched by 0.8‰. $\delta^{13}C$ values do not follow a similar theoretical relationship, but can be predicted if the isotopic composition of C sources (atmospheric CO_2, experimental CO_2, and seawater DIC) are known, using equilibrium carbon isotopic fractionation factors between these sources, assuming carbonate system equilibrium conditions.

Results

Seawater and inorganic carbon parameters for the tanks are summarized in Table 1. As reported in Knorr *et al.* (2015), there were no statistically significant differences in the measured temperatures,

Table 1. Carbonate system parameters that were analysed during experiment.

Seawater parameter	*N*	pH control	*N*	pH experimental
pH_{tot}	18	8.0 (± 0.01)	18	7.61 (± 0.01)
DIC (µmol kg_{sw}^{-1})	18	2061.8	18	2228.3
ALK (µmol kg_{sw}^{-1})	18	2305 (± 8)	18	2309 (± 5)
pCO_2 (µatm)	18	466.7	18	1290.2
$\Omega_{calcite}$	18	4.4	18	2.01
Temperature °C	18	23.9 (± 0.1)	18	23.9 (± 0.12)
Salinity	18	31.8 (± 0.2)	18	32.1 (± 0.5)

Mean (± s.d.) seawater parameters in treatments during the 6-week experiment.
Values for DIC, pCO_2, and $\Omega_{calcite}$ were calculated using CO2calc (version 1.3) (Robbins *et al.*, 2010) using pH—alkalinity data pair, carbonic acid dissociation constants from Lueker *et al.* (2000), HSO_4^- constants from Dickson (1990), and borate dissociation constant of Uppstrom (1974).

Table 2. Oxygen and carbon stable isotopic values for adult (terminal chamber analysed and whole test) and juvenile (whole test) LBF at pH 8.0 and 7.6 (average ± s.d.).

		pH 8.0				pH 7.6			
Species	**Sample type**	***N***	**$\delta^{18}O$**	***N***	**$\delta^{13}C$**	***N***	**$\delta^{18}O$**	***N***	**$\delta^{13}C$**
A. gibbosa	Adult whole test	10	−0.03 ± 0.20	10	−1.29 ± 0.52	10	−0.89 ± 0.34	10	−1.08 ± 0.62
	Adult terminal chamber	8	−0.51 ± 0.43	8	−1.39 ± 0.64	8	−0.42 ± 0.51	8	−1.42 ± 0.23
	Juvenile whole test	7	−1.10 ± 0.09	7	−1.86 ± 0.32	7	−0.37 ± 0.10	7	0.57 ± 0.25
A. angulatus	Adult whole test	12	0.22 ± 0.46	12	1.33 ± 1.04	13	−0.02 ± 0.34	13	1.85 ± 0.38
	Adult terminal chamber	21	0.61 ± 0.91	21	1.51 ± 0.44	15	1.05 ± 0.25	15	1.50 ± 0.36
	Juvenile whole test	8	−0.80 ± 0.15	8	1.26 ± 0.26	8	0.08 ± 0.06	8	2.14 ± 0.26

Table 3. Results of ANOVA GLM of *A. gibbosa* oxygen and carbon isotopes.

Source	**D.F.**	***F*-value**	***p*-value**
Oxygen isotope GLM			
pH treatment (8.0, 7.6)	1	13.42	0.001
Sample type[a] (pH treatment)	4	8.44	0.000
Tank replicates (sample type, pH treatment)	15	2.40	0.122
Carbon isotope GLM			
pH treatment (8.0, 7.6)	1	30.96	0.00
Sample type[a] (pH treatment)	4	13.39	0.000
Tank replicates (sample type, pH treatment)	7	2.09	0.069

[a]Sample type refers to juvenile, terminal chamber adult, and whole chamber samples.

Table 4. Results of ANOVA GLM of *A. angulatus* oxygen and carbon isotopes.

Source	**D.F.**	***F*-value**	***p*-value**
Oxygen isotope GLM			
pH treatment (8.0, 7.6)	6	3.89	0.002
Sample type[a] (pH treatment)	5	10.08	0.000
Tank replicates (sample type, pH treatment)	2	0.24	0.787
Carbon isotope GLM			
pH treatment (8.0, 7.6)	6	1.16	0.341
Sample type[a] (pH treatment)	4	0.44	0.816
Tank replicates (sample type, pH treatment)	2	0.61	0.548

[a]Sample type refers to juvenile, terminal chamber adult, and whole chamber samples.

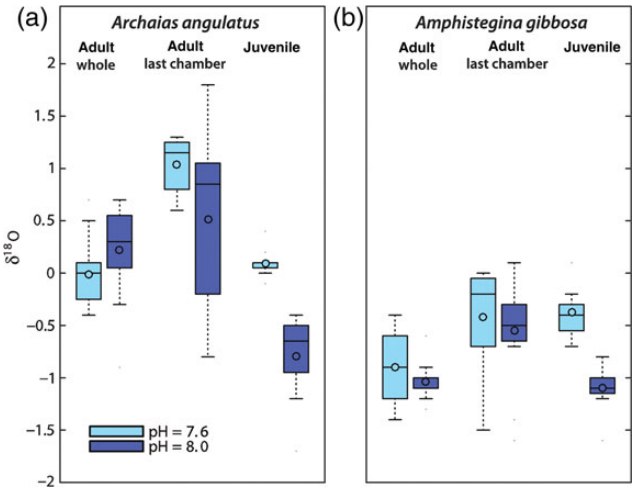

Figure 5. (a and b) Box-and-whisker plots of $\delta^{18}O$ values (VPDB scale) from adult and juvenile specimens of *A. angulatus* and *A. gibbosa* cultured at pH 8.0 and 7.6. Boxes show 1st–3rd quartiles with central line at median values; whiskers show the range of values; circles show mean value.

salinity, or alkalinity ($n = 54$, $p = 0.984$, 0.963, and 0.996, respectively) between control and experimental tanks. Neither the control tanks (pH 8.0) nor the experimental tanks (pH 7.6) showed significant intra-tank differences in measured pH ($n = 27$, $p = 0.493$ and 0.997, respectively) or calculated calcite saturation state (Ω_c) ($n = 27$, $p = 0.992$ and 0.995, respectively). The mean $\delta^{18}O$ of the tank seawater (1.6 ± 0.2‰; VSMOW scale) differed from that of a sample from Dolphin Cove (−4.0‰ on VSMOW scale). Likewise, mean $\delta^{13}C$ values of the tank water (−3.0 ± 0.2‰; VPDB scale) differed from that of the sample from Dolphin Cove (−1.6‰ on VPDB scale).

Mean isotopic values for adult whole tests, adult terminal chambers, and juvenile whole tests for *A. gibbosa* and *A. angulatus* in the control and experimental tanks after the 6-week experiment are displayed in Table 2. Tukey's pairwise comparisons revealed that $\delta^{18}O$ values of adult whole tests were significantly different from adult terminal chambers for both *A. gibbosa* and *A. angulatus* (VPDB scale; adjusted $p < 0.001$), but not for $\delta^{13}C$ values (VPDB scale, $p > 0.5$) at the 98% confidence level. The main effects of pH treatment, type of sample, and tank replicates on isotopic composition are presented in Tables 3 and 4, where the pH treatment (by sample type and tank) and sample type (by tank) are significantly related to oxygen and carbon isotopic composition for *A. gibbosa* and oxygen isotopic composition for *A. angulatus*. As previously indicated by other tests, the replicates are not significantly different.

Experimental vs. control

Adult whole-test analysis of *A. angulatus* showed little difference between the experimental and control pH on $\delta^{18}O$ values (VPDB; $p < 0.62$) with a lack of significance for $\delta^{13}C$ values ($p > 0.9$). The $\delta^{18}O$ and $\delta^{13}C$ values for adult terminal chambers of *A. angulatus* were insignificant between experimental pH and controls (VPDB; Figures 5a and 6a; $n = 41$, $p > 0.3$ and 1.0, respectively). No significant differences ($p > 0.99$) were observed between experimental (pH 7.6) means and control mean $\delta^{18}O$ (Figure 5b) or $\delta^{13}C$ values (Figure 6b) for either whole tests or adult terminal chamber of *A. gibbosa* (Figure 6b). Juveniles of both species exhibited significant differences in mean $\delta^{18}O$ values between pH treatments ($p < 0.005$ for both; Figure 5a and b). In contrast to juvenile *A. angulatus*, which did not show significant differences for $\delta^{13}C$ values between experimental and control conditions ($p = 0.25$, Table 4), juvenile

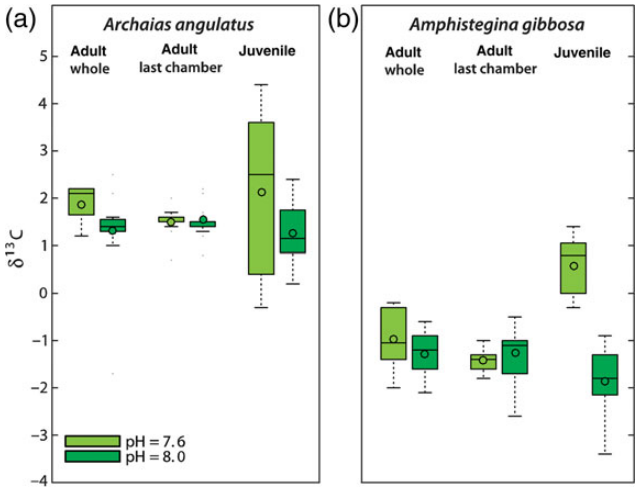

Figure 6. (a and b) Box-and-whisker plots of $\delta^{13}C$ values (VPDB scale) from adult and juvenile specimens of *A. angulatus* and *A. gibbosa* cultured at different pH 8.0 and 7.6. Boxes show 1st – 3rd quartiles with central line at median values; whiskers show the range of values; circles show mean value.

A. gibbosa displayed highly significant difference ($p < 0.001$) for mean $\delta^{13}C$ values between experimental and control tanks (Figure 6b).

Discussion

Both equilibrium and disequilibrium kinetic effects on isotopes of inorganic carbon species have been shown to contribute to the total observed fractionation of carbon and oxygen isotopes between $CO_{2(aq)}$ and the anions involved in carbonate precipitation, $[HCO_3^-]$ and $[CO_3^{2-}]$ (Spero *et al.*, 1997; Zeebe, 1999). At equilibrium, $[CO_3^{2-}]$ is ^{18}O-depleted by as much as 16‰ relative to $[HCO_3^-]$, and $[HCO_3^-]$ is ^{18}O-depleted by 24‰ relative to $[CO_{2(aq)}]$. Thus, as pH decreases the $[CO_3^{2-}]$: $[HCO_3^-]$ ratio, the carbon speciation effect increases both $\delta^{18}O$ and $\delta^{13}C$ values. Carbon isotopic composition is subject to metabolic and abiotic kinetic fractionation as well as relatively small temperature-dependent equilibrium fractionation factors, whereas oxygen isotopic composition primarily reflects large equilibrium fractionation factors and variation in the total fractionation with inorganic carbon speciation (Spero *et al.*, 1997). Rotalids, such as *A. gibbosa*, store ions from multiple sources in an internal carbon pool for use in calcification (Erez, 2003), while miliolids, such as *A. angulatus*, precipitate calcite within vesicles containing relatively unaltered seawater (ter Kuile and Erez, 1988). Therefore, distinct speciation effects on stable isotopic composition are predicted to follow these different metabolic processes; the results of this experiment reflect these divergent calcification mechanisms.

Isotopic disequilibrium that arises during precipitation of LBF carbonate tests can be caused by respiration, ontogenetic effects, symbiont photosynthesis, and other vital effects (Wefer and Berger, 1991; Bemis *et al.*, 1998; Rohling and Cooke, 2003). Previously reported stable isotopic values for modern *A. angulatus* tests from natural environments range between approximately −1.0 and +1.0‰ for $\delta^{18}O$, and between +1.0 and +4.5‰ for $\delta^{13}C$ (VPDB scale); specimens from the same location can exhibit variations of ∼1.0‰ (Gross, 1964; Wefer, 1985; Brasier and Green, 1993; Knorr *et al.*, 2015; VPDB scale). Moreover, stable isotopic composition for *Amphistegina* spp. can vary between approximately −3.5 and −1.0‰ for $\delta^{18}O$ and approximately −2.0 and +2.0‰ for $\delta^{13}C$ (Vinot-Bertouille and Duplessy, 1973; Saraswati *et al.*, 2004; Rollion-Bard *et al.*, 2008; Knorr, 2014; VPDB scale). Intraspecies variations of >2‰ are common (Vinot-Bertouille and Duplessy, 1973), and little or no correlation between $\delta^{18}O$ and $\delta^{13}C$ values has been observed (Saraswati *et al.*, 2004).

Stable isotopic responses of LBF

Studies of benthic foraminifera have documented varied isotopic responses to changes in pH and pCO_2. *Amphistegina* spp. follow the predicted speciation effect of decreased $\delta^{18}O$ values with increased pH and $[CO_3^{2-}]$ (Rollion-Bard *et al.*, 2008). Similarly, the culture study of the symbiotic planktonic foraminifer *Orbulina universa* d'Orbigny by Spero *et al.* (1997) was the first to demonstrate the speciation effect, finding that at that lower pH, the decrease in ^{18}O-depleted $[CO_3^{2-}]$ and increase in ^{18}O-enriched $[HCO_3^-]$, resulted in lower $\delta^{18}O$ (and $\delta^{13}C$) values in cultured foraminifera.

The analytical results on the juvenile specimens presented here agree with previous work on the relationship between carbonate speciation and oxygen isotopic fractionation (Spero *et al.*, 1997; Zeebe, 1999; McConnaughey, 2003). Specifically, analyses of juvenile tests demonstrate the relative enrichment of $\delta^{18}O$ values in the lower pH treatment (7.6) relative to the control (8.0) of both rotalids (∼0.7‰) and miliolids (∼0.9‰), consistent with the 0.8‰ ^{18}O-enrichment predicted by the mass balance calculation of equilibrium speciation effects (Zeebe, 1999). The agreement between observed and predicted $\delta^{18}O$ values of the juvenile tests indicates that equilibrium fractionation plays the dominant role in determining the $\delta^{18}O$ values, with little or no variation in the biological kinetic ^{18}O-discrimination involved in the selection of ions for use in calcification. In contrast, neither whole adult tests nor terminal chambers show the expected oxygen isotopic enrichment (Figure 5a and b) after 6 weeks at low pH treatment.

Miliolids, including those with algal symbionts such as *A. angulatus*, precipitate carbon directly from seawater (ter Kuile and Erez, 1988). In contrast, rotalids produce internal carbon pools containing carbon derived from seawater, algal endosymbiont photosynthesis, and metabolic processes (de Nooijer *et al.*, 2014). The invariance of miliolid $\delta^{13}C$ values in different pH treatments can be attributed to the lack of a relationship between pH and the $\delta^{13}C$ of dissolved inorganic carbon species of seawater, and the subsequent direct precipitation of carbonate, with no significant biological isotopic effects.

The significant difference found in the $\delta^{13}C$ of juvenile *A. gibbosa* from different pH treatments may be the result of a vital effect. Growth in *A. gibbosa* is stimulated at modestly elevated levels of $[CO_{2atm}]$ (Knorr *et al.*, 2015). Their diatom endosymbionts can benefit from increased concentrations of CO_2 and HCO_3^-, a consequence of the lower seawater pH associated with OA (Engel *et al.*, 2015). The experimental results of the carbon isotopic composition (Figure 6b) indicate that *A. gibbosa's* internal carbon pool is relatively ^{13}C-enriched under low pH conditions, a likely consequence of preferential uptake of ^{12}C for photosynthesis by the diatom symbionts. However, the adult *A. gibbosa* specimens (both whole test and terminal chamber values) do not show the expected carbon isotopic enrichment (Figure 6a and b); this result is discussed below.

The results of this experiment indicate that the $\delta^{18}O$ values of both rotalid and miliolid LBF are dependent on the carbonate

speciation effects in the surrounding seawater; this speciation is primarily dependent on pH (Pilson, 1998). However, the relationship of seawater pH and test $\delta^{13}C$ values is more nuanced; miliolid $\delta^{13}C$ values are independent of pH, whereas rotalid $\delta^{13}C$ values are influenced by pH. Comparing the results from representatives of two very different foraminiferal lineages, in which biomineralization appears to have independently evolved (Pawlowski *et al*., 2013; Mikhalivich, 2014), provides insights into how different taxa may respond to changes in the marine carbonate system and confirm using stable oxygen isotopes use in paleoenvironmental reconstruction of paleo pH. Furthermore, the importance of appropriate selection of laboratory specimens becomes apparent when stable isotopic values of adult whole tests, adult terminal chamber, and juveniles are compared.

Adults vs. juvenile specimens

Observed differences in oxygen isotopic speciation between adult (both whole test and terminal chamber) and juvenile tests likely reflect the fact that the adult specimens originated from field samples, so early chambers precipitated in equilibrium with isotopic composition of their harvest localities in the Florida Keys, while juveniles calcified only under experimental conditions (Table 1). At the conclusion of the experiment, preparation of the adult foraminifera for analysis involved dissection of terminal chambers, which were presumed to have been precipitated during the experiment. Since labelling the original chambers was not performed, we cannot be completely confident that the sampling of the adults only included those chambers precipitated during the experiment, despite the use of photographs to document each individual and their growth periodically over 6 weeks, which was then used as a guide for terminal chamber dissection. More likely, however, bias may have been introduced as a function of dissection of the terminal chambers, since precise extraction of individual chambers from the others was impossible. This problem is particularly apparent for *A. gibbosa*, in which a new layer of calcite is deposited over previously formed ones with each chamber addition. Not only might dissected chambers have pieces of chambers produced earlier in ontogeny still attached, which could bias the isotopic results, but also the deposition of new calcite would alter the signal from the older part of the test. Potential contamination may be more prevalent depending on the original size of the test with more original chambers increasing that potential, subsequent growth rate of chambers onto previously existing chambers during the treatment period, and duration of the experiment. More precise analysis of individual chambers may be feasible with smaller analytical sample sizes (tens of μg), and in particular using new micro-analytical approaches such as secondary ion mass spectrometry (Vetter *et al*., 2013, 2014).

In our experiments, the difference in $\delta^{18}O$ values of the native seawater and tank experiments was 4.6‰ and the averaging of that was seen when comparing the adults with the juveniles. This indicates that if whole, mature specimens are selected for calcification experiments, the growth of only one or two chambers during the pH treatment is insufficient to dilute the original stable isotopic signals. As a result, our data indicate that isotopic analyses of juvenile specimens produced during an OA experiment are more likely to reveal differences than analyses of adult LBF that originated from field samples or stock cultures.

Conclusions

Can stable isotopes in LBF be used as a proxy for OA? The effects of lowered seawater pH on the stable oxygen and carbon isotopes of two prolific sediment-producing species of foraminifera demonstrated distinct enrichments in stable oxygen isotopes of juveniles produced under low pH conditions. Moreover, isotopic results from the culture experiments using adult specimens, or even dissected "last chambers/terminal chambers" of adult specimens, are likely to be of limited use because of overlapping test construction.

However, stable isotopic analysis of juvenile foraminifera grown under low pH conditions responded as expected, showing an enrichment in both $\delta^{18}O$ and $\delta^{13}C$ values when compared with control values. As CO_{2atm} approaches 1300, our data suggest that the lower pH associated with OA will more significantly impact the miliolids, such as *A. angulatus*. Further knowledge of the calcification mechanisms of different species, especially how carbon and oxygen are incorporated during the biomineralization of a foraminiferal test, is critical in interpreting results.

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References

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

BouDagher-Fadel, M. 2008. Evolution and Geological Significance of Larger Benthic Foraminifera. Elsevier, Amsterdam. 544 pp.

Brasier, M. D., and Green, O. R. 1993. Winners and losers: Stable isotopes and microhabitats of living Archaiadae and Eocene Nummulites (larger foraminifera). Marine Micropaleontology, 20: 267–276.

Caldeira, K., and Wickett, M. E. 2003. Anthropogenic carbon and ocean pH. Nature, 425: 365.

Clayton, T. D., and Byrne, R. H. 1993. Spectrophotometric seawater pH measurements: Total hydrogen ion concentration scale calibration of m-cresol purple and at-sea results. Deep Sea Research, 40: 2115–2129.

Cottey, T. L., and Hallock, P. 1988. Test surface degradation in *Archaias angulatus*. Journal of Foraminiferal Research, 18: 187–202.

de Nooijer, L. J., Spero, H. J., Erez, J., Bijma, J., and Reichart, G. J. 2014. Biomineralization in perforate Foraminifera. Earth-Science Reviews, 135: 48–58.

de Nooijer, L. J., Toyofuku, T., and Kitazato, H. 2009. Foraminifera promote calcification by elevating their intracellular pH. Proceedings of the National Academy of Sciences, 106: 15374–15378.

Dettmering, C., Röttger, R., Hohenegger, J., and Schmalijohann, R. 1998. The trimorphic life cycle in Foraminifera: Observations from cultures allow new evaluation. European Journal of Protistology, 34: 363–368.

Dickson, A. G. 1990. Standard potential of the reaction $AgCl(s)+5H_2(g)=Ag(s)+HCl(aq)$ and the standard acidity constant of the ion HSO_4^- in synthetic sea water from 273.15 to 318.15 K. The Journal of Chemical Thermodynamics, 22: 113–127.

Dickson, A. G., Afghan, J. D., and Anderson, G. C. 2003. Reference materials for oceanic CO_2 analysis: A method for the certification of total alkalinity. Marine Chemistry, 80: 185–197.

Dickson, A. G., Sabine, C. L., and Christian, J. R. (Eds.) 2007. Guide to Best Practices for Ocean CO_2 Measurements. PICES Special Publication 3, 191 pp.

Doney, S. C., Fabry, V. J., Feely, R. A., and Kleypas, J. A. 2009. Ocean acidification: The other CO_2 problem. Annual Review of Marine Science, 1: 169–192.

Doo, S. S., Fujita, K., Byrne, M., and Uthicke, S. 2014. Fate of calcifying tropical symbiont-bearing large benthic Foraminifera: Living sands in a changing ocean. Biological Bulletin, 226: 169–186.

Emiliani, C. 1955. Pleistocene temperatures. The Journal of Geology, 63: 538–578.

Engel, B. E., Hallock, P., Price, R. E., and Pichler, T. 2015. Shell dissolution in larger benthic foraminifers exposed to pH and temperature extremes: Results from an in situ experiment. Journal of Foraminiferal Research, 45: 190–203.

Erez, J. 2003. The source of ions for biomineralization in Foraminifera and their implications for paleoceanographic proxies. Reviews in Mineralogy and Geochemistry, 54: 115–149.

Fichtel, L., and Moll, J. P. C. 1798. Testacea Microscopica Aliaque minuta ex Generibus. Argonauta et Nautilus, ad Naturam Picta et Descript. Camesina, Vienna. 190 pp.

Gross, M. G. 1964. Variations in the O18/O16 and C13/C12 ratios of diagenetically altered limestones in the Bermuda Islands. The Journal of Geology, 72: 170–194.

Grossman, E. L. 1987. Stable isotopes in modern benthic foraminifera: a study of vital effect. Journal of Foraminiferal Research, 17: 48–61.

Hallock, P. 1981. Production of carbonate sediments by selected Foraminifera on two Pacific coral reefs. Journal of Sedimentary Petrology, 51: 467–474.

Hallock, P., Cottey, T. L., Forward, L. B., and Halas, J. 1986a. Population biology and sediment production of *Archaias angulatus* (Foraminiferida) in Largo Sound, Florida. Journal of Foraminiferal Research, 16: 1–18.

Hallock, P., Forward, L. B., and Hansen, H. J. 1986b. Influence of environment on the test shape of *Amphistegina*. Journal of Foraminiferal Research, 16: 224–231.

Hemleben, C., Anderson, O. R., Berthold, W., and Spindler, M. 1986. Calcification and chamber formation in Foraminifera—A brief overview. *In* Biomineralization in Lower Plants and Animals, pp. 237–249. Ed. by R. Riding, and B. S. C. Leadbeater. Clarendon Press, Oxford University Press, Oxford.

Imbrie, J., Hays, J. D., Martinson, D. G., McIntyre, A., Mix, A. C., Morley, J. J., Pisias, N. G. *et al.* 1984. The orbital theory of Pleistocene climate: Support from a revised chronology of the marine delta ^{18}O record. *In* Milankovitch and Climate: Understanding the Response to Astronomical Forcing, pp. 1–269. Ed. by A. L. Berger, J. Imbrie, J. Hays, G. Kukla, and B. Saltzman Springer, Netherlands.

Keul, N., Langer, M. G., de Nooijer, L. J., and Bijma, J. 2013. Effect of ocean acidification on the benthic Foraminifera *Ammonia* sp. is caused by a decrease in carbonate ion concentration. Biogeosciences, 10: 6185–6198.

Knorr, P. O. 2014. Response of Benthic Foraminifera to Ocean Acidification and Impact on Florida's Carbonate Sediment Production. School of Geosciences, University of South Florida, Tampa, FL. 166 pp.

Knorr, P. O., Robbins, L. L., Harries, P. J., Hallock, P., and Wynn, J. 2015. Response of the miliolid *Archaias angulatus* to simulated ocean acidification. Journal of Foraminiferal Research, 45: 109–127.

Kroeker, K. J., Kordas, R. L., Crim, R., and Singh, G. S. 2010. Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. Ecology Letters, 13: 1419–1434.

Langer, M. R. 2008. Assessing the contribution of foraminiferan protists to global ocean carbonate production. Journal of Eukaryotic Microbiology, 55: 163–169.

Langer, M. R., Silk, M. T., and Lipps, J. H. 1997. Global ocean carbonate and carbon dioxide production; The role of reef Foraminifera. Journal of Foraminiferal Research, 27: 271–277.

Lee, J. J., Crockett, L. J., Hagen, J., and Stone, R. J. 1974. The taxonomic identity and physiological ecology of *Chlamydomonas hedleyi* sp. nov., algal flagellate symbiont from the foraminifer *Archaias angulatus*. British Phycological Journal, 9: 407–422.

Lowenstam, H. A., and Weiner, S. 1989. On Biomineralization. Oxford University Press, New York. 324 pp.

Lueker, T. J., Dickson, A. G., and Keeling, C. D. 2000. Ocean pCO_2 calculated from dissolved inorganic carbon, alkalinity, and equations for K1 and K2—Validation based on laboratory measurements of CO_2 in gas and seawater at equilibrium. Marine Chemistry, 70: 105–119.

Lynts, G. W., and Pfister, R. M. 1967. Surface ultrastructure of some tests of recent Foraminiferida from the Dry Tortugas, Florida. Journal of Eukaryotic Microbiology, 14: 387–399.

McConnaughey, T. A. 2003. Sub-equilibrium oxygen-18 and carbon-13 levels in biological carbonates: Carbonate and kinetic models. Coral Reefs, 22: 316–327.

McCrea, J. M. 1950. On the isotopic chemistry of carbonates and a paleotemperature scale. Journal of Chemical Physics, 18: 849.

McIntyre-Wressnig, A., Bernhard, J. M., McCorkle, D. C., and Hallock, P. 2013. Non-lethal effects of ocean acidification on the symbiont-bearing benthic Foraminifera *Amphistegina gibbosa*. Marine Ecology Progress Series, 472: 45–60.

Mikhalivich, V. I. 2014. Post-Cambrian Testate Foraminifera as a System in its Evolution. Nova Publishers, New York, 426 pp.

Pawlowski, J., Holzmann, M., and Tyszka, J. 2013. New supraordinal classification of Foraminifera: Molecules meet morphology. Marine Micropaleontology, 100: 1–10.

Pilson, M. E. Q. 1998. An Introduction to the Chemistry of the Sea. Cambridge University Press, Cambridge. 431 pp.

Pörtner, H-O., Karl, D. M., Boyd, P. W., Cheung, W. W. L., Lluch-Cota, S. E., Nojiri, Y., Schmidt, D. N., *et al.* 2014. Ocean systems. *In* Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, pp. 411–484. Ed. by C. B. Field, V. R. Barros, D. J. Dokken, K. J. Mach, M. D. Mastrandrea, T. E. Bilir, M. Chatterjee, *et al.* Cambridge University Press, Cambridge, UK and New York, NY, USA.

Prazeres, M., Uthicke, S., and Pandolfi, J. M. 2015. Ocean acidification induces biochemical and morphological changes in the calcification process of large benthic Foraminifera. Proceedings of the Royal Society B: Biological Sciences, 282(1803). doi:10.1098/rspb.2014.2782.

Reid, R. P., and Macintyre, I. G. 1998. Carbonate recrystallization in shallow marine environments: A widespread diagenetic process forming micritized grains. Journal of Sedimentary Research, 68: 928–946.

Robbins, L. L., Hansen, M. E., Kleypas, J. A., and Meylan, S. C. 2010. CO2calc—A User-friendly Seawater Carbon Calculator for Windows, Max OS X, and iOS (iPhone). US Geological Survey Open-File Report 2010–1280. 17 pp.

Rohling, E. J., and Cooke, S. 2003. Stable oxygen and carbon isotopes in foraminiferal carbonate shells. *In* Modern Foraminifera, pp. 239–258. Ed. by B. Sen Gupta. Springer, Netherlands.

Rollion-Bard, C., Erez, J., and Zilberman, T. 2008. Intra-shell oxygen isotope ratios in the benthic Foraminifera genus *Amphistegina* and

the influence of seawater carbonate chemistry and temperature on this ratio. Geochimica et Cosmochimica Acta, 72: 6006–6014.

Saraswati, P. K., Seto, K., and Nomura, R. 2004. Oxygen and carbon isotopic variation in co-existing larger Foraminifera from a reef flat at Akajima, Okinawa, Japan. Marine Micropaleontology, 50: 339–349.

Shackleton, J., and Opdyke, N. D. 1973. Oxygen isotope and paleomagnetic stratigraphy of Equatorial Core V28–238: Oxygen isotope temperature and ice volumes on a 105 and a 106 year scale. Quaternary Research, 3: 39–55.

Shackleton, N. J. 1977. Carbon-13 in Uvigerina: Tropical rainforest history and the equatorial Pacific carbonate dissolution cycles. *In* The Fate of Fossil Fuel CO_2 in the Oceans, pp. 401–427. Ed. by N. R. Andersen, and A. Malahoff Plenum Press, New York.

Spero, H. J., Bijma, J., Lea, D. W., and Bemis, B. E. 1997. Effect of seawater carbonate concentration on foraminiferal carbon and oxygen isotopes. Nature, 390: 497–499.

Stocker, T. F. 2013. The ocean as a component of the climate system. *In* Ocean Circulation and Climate: A 21st Century Perspective, 2nd edn, pp. 3–30. Ed by G. Siedler, *et al.* Academic Press, Oxford.

Talge, H. K., and Hallock, P. 1995. Cytological examination of symbiont loss in a benthic foraminifera, *Amphistegina gibbosa*. Marine Micropaleontology, 26: 107–113.

Talge, H. K., and Hallock, P. 2003. Ultrastructural responses in field-bleached and experimentally stressed *Amphistegina gibbosa* (class Foraminifera). Journal of Eukaryotic Microbiology, 50: 324–333.

ter Kuile, B., and Erez, J. 1988. The size and function of the internal inorganic carbon pool of the foraminifer *Amphistegina lobifera*. Marine Biology, 99: 481–487.

ter Kuile, B., Erez, J., and Padan, E. 1989. Mechanisms for the uptake of inorganic carbon by two species of symbiont-bearing Foraminifera. Marine Biology, 103: 241–251.

Uppstrom, L. R. 1974. Boron/chlorinity ratio of deep-sea water from the Pacific Ocean. Deep Sea Research, 21: 161–162.

Uthicke, S., and Fabricius, K. E. 2012. Productivity gains do not compensate for reduced calcification under near-future ocean acidification in the photosynthetic benthic foraminifer species *Marginopora vertebralis*. Global Change Biology, 18: 2781–2791.

Vetter, L., Kozdon, R., Mora, C. I., Eggins, S. M., Valley, J. M., Hönisch, B., and Spero, H. J. 2013. Micron-scale intrashell oxygen isotope variation in cultured planktic foraminifers. Geochimica et Cosmochimica Acta, 107: 267–278.

Vetter, L., Kozdon, R., Valley, J. W., Mora, C. I., and Spero, H. J. 2014. SIMS measurements of intrashell $\delta^{13}C$ values in the cultured planktic foraminifer *Orbulina universa*. Geochimica et Cosmochimica Acta, 139: 527–539.

Vinot-Bertouille, A. C., and Duplessy, J-C. 1973. Individual isotopic fractionation of carbon and oxygen in benthic Foraminifera. Earth and Planetary Science Letters, 18: 247–252.

Wefer, G. 1985. Die Verteilung Stabiler Isotope in Kalkschalen Mariner Organismen. Geologisches Jahrbuch, Bundesanstalt für Geowissenschaften und Rohstoffe, Reihe A, Heft, 82: 3–111.

Wefer, G., and Berger, W. H. 1991. Isotope paleontology: Growth and composition of extant calcareous species. Marine Geology, 100: 207–248.

Weiner, S., and Dove, P. M. 2003. An overview of biomineralization processes and the problem of the vital effect. Reviews in Mineralogy and Geochemistry, 54: 1–29.

Wetmore, K. L. 1999. Chamber formation in *Archaias angulatus*. Journal of Foraminiferal Research, 29: 69–74.

Yao, W., and Byrne, R. H. 1998. Simplified seawater alkalinity analysis: Use of linear array spectrometers. Deep Sea Research Part I, 45: 1383–1392.

Zeebe, R. E. 1999. An explanation of the effect of seawater carbonate concentration on foraminiferal oxygen isotopes. Geochimica et Cosmochimica Acta, 63: 2001–2007.

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