

A culture-based calibration of benthic foraminiferal paleotemperature proxies: $\delta^{18}\text{O}$ and Mg/Ca results

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Abstract. Benthic foraminifera were cultured for five months at four temperatures (4, 7, 14 and 21 °C) to establish the temperature dependence of foraminiferal calcite $\delta^{18}\text{O}$ and Mg/Ca. Two *Bulimina* species (*B. aculeata* and *B. marginata*) were most successful in terms of calcification, adding chambers at all four temperatures and reproducing at 7 and 14 °C. Foraminiferal $\delta^{18}\text{O}$ values displayed ontogenetic variations, with lower values in younger individuals. The $\delta^{18}\text{O}$ values of adult specimens decreased with increasing temperature in all but the 4 °C treatment, exhibiting a relationship consistent with previous $\delta^{18}\text{O}$ paleotemperature calibration studies. Foraminiferal Mg/Ca values, determined by laser ablation inductively coupled plasma mass spectrometry, were broadly consistent with previous Mg/Ca calibration studies, but extremely high values in the 4 °C treatment and higher than predicted values at two of the other three temperatures make it challenging to interpret these results.

al., 1997; Lear et al., 2000). Most foraminiferal paleochemical and paleotemperature reconstructions rely on modern-ocean empirical calibrations, which are based on core-top sediments, but the inherent environmental variability in the ocean, and the co-variation of many important environmental, ecological, and physiological factors in the ocean can complicate interpretation of these empirical calibrations. An alternative way to investigate the controls on foraminiferal geochemical proxies is to grow the foraminifera in the laboratory under known environmental conditions.

Culturing studies using planktonic foraminifera provide well-known examples of this approach (e.g., Erez and Luz, 1982; Bijma et al., 1990; Nürnberg et al., 1996; Lea and Spero, 1992; Spero et al., 1997; Bemis et al., 1998; Bijma et al., 1999). Culturing studies also have been used to investigate the stable oxygen and carbon isotopic composition (Chandler et al., 1996; Wilson-Finelli et al., 1998; McCorkle et al., 2008; Barras et al., 2010) and trace metal content (Russell et al., 1994; Toyofuku et al., 2000; Toyofuku and Kitazato, 2005; Havach et al., 2001; Segev and Erez, 2006; Hintz et al., 2006a, b) of benthic foraminifera. Here, we used a single seawater reservoir to culture benthic foraminifera at four different temperatures. Because microhabitat effects were minimized and the water chemistry kept constant across the range of temperatures, our culture experiment addresses key uncertainties in the existing, field-based calibrations of benthic foraminiferal $\delta^{18}\text{O}$ and Mg/Ca.

1 Introduction

Quantitative paleoceanographic reconstructions are an essential component of efforts to understand the oceans' role in climate, to document natural climate variability on a range of time scales, and to predict the impact of future climate change. The isotopic and elemental compositions of the calcium carbonate tests (shells) of fossil foraminifera are widely used to estimate ocean chemistry and temperature at the time of calcification (e.g., Shackleton et al., 1984; Rosenthal et



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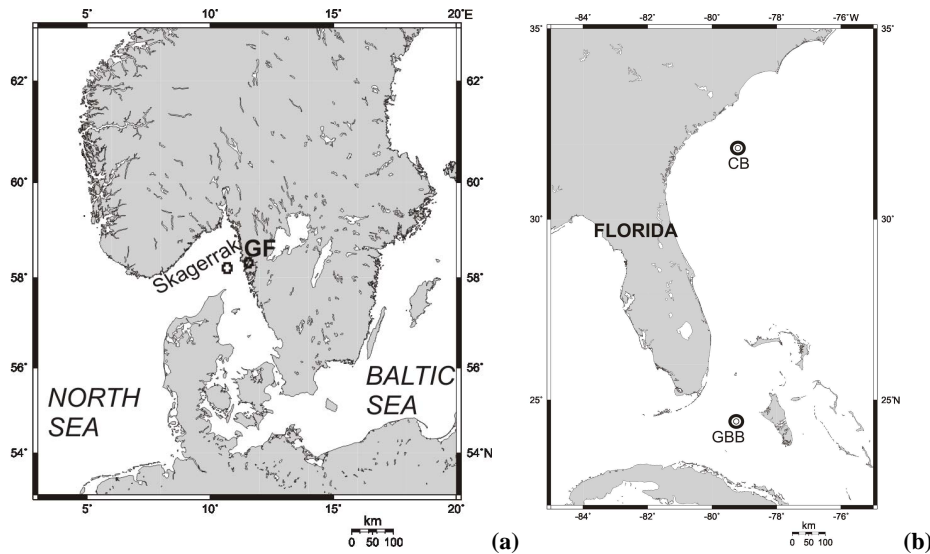


Fig. 1. Figure 1a Map showing sampling locations, indicated with crosses, in the NE Skagerrak and Gullmar Fjord (GF). Figure 1b. Sampling locations indicated with circles at Charleston Bump (CB) and Great Bahama Bank (GBB). Map from www.aquarius.ifm-geomar.de

Table 1. Sampling details from the sites in the North Atlantic.

Site	Date	Water depth (m)	Water T ($^{\circ}\text{C}$)	Corer	Screen size (μm)
Gullmar Fjord	8/2006	118	6	Gemini	63
Charleston Bump	5/2006	~220	9	Soutar boxcorer	90, 125, 500
Skagerrak	8/2006	330	6.7	Gemini	63
Adjacent to Great Bahama Bank	5/2006	466–814	8–10	Soutar boxcorer	90, 125, 500

2 Methods

2.1 Collection and maintenance

Living foraminifera were obtained by coring the seafloor at various locations and transporting surface sediments (top ~2 cm) to the laboratory at Woods Hole Oceanographic Institution (WHOI), USA. Samples were collected from the northeastern Skagerrak (330 m), Gullmar Fjord, Sweden (118 m), the Charleston Bump off South Carolina, USA (~220 m), and at three sites near the Great Bahama Bank (466–814 m) (Fig. 1, Table 1). Bottom water was collected at each site using a CTD-Niskin rosette, maintained at near bottom-water temperatures, and used for sieving to remove fine material and concentrate the benthic foraminifera. Immediately after sieving, the samples were transferred to a plastic container and stored at 5–7 $^{\circ}\text{C}$ until the samples were transported in coolers to WHOI after the cruise.

In our shore-based 7 $^{\circ}\text{C}$ environmental room, the sample containers were plumbed into a recirculating seawater system, designed to maintain stock cultures of benthic foraminifera (Chandler and Green, 1996). The foraminifera were fed weekly with a mixture of *Dunaliella tertiolecta* and

Isochrysis galbana (Hintz et al., 2004). Subsamples were taken from the stock cultures and sieved, using chilled artificial sea water, over a 63- μm screen. Specimens showing signs of activity, by containing algae or having colored cytoplasm, were picked from the >63- μm fraction and incubated at 7 $^{\circ}\text{C}$ in Petri dishes with artificial sea water and calcein (10 mg/l) solution for two to four weeks, following Bernhard et al. (2004). The samples were fed every week and the calcein solution was changed every 3–4 days.

Just before initiation of the experiment, specimens were examined using epifluorescence microscopy at 480-nm excitation and 520-nm emission, and fluorescent specimens, which had precipitated calcite during the calcein incubation, were picked using a fine brush or pipette. All examination, sorting, and picking were carried out on ice. Fluorescent specimens were transferred to cell tissue culture cups (8-ml volume; 8- μm nominal pore diameter) and placed in acrylic culture chambers (Hintz et al., 2004). Each culture cup contained a 2–3 mm thick layer of HPLC-grade silt-sized silica to provide a substrate for the foraminifera.

Most culture chambers were inoculated with a mix of species, with all inoculates in any given culture chamber from the same sampling site. The exceptions were three

“monoculture” chambers that contained a mix of *Bulimina aculeata* and *B. marginata* from Charleston Bump and adjacent to the Great Bahama Bank (see below). Skagerrak species included *Bolivina skagerrakensis*, *Bolivinellina pseudopunctata*, *Bulimina marginata*, *Cassidulina laevigata*, *Globobulimina turgida*, *Hyalinea balthica*, *Melonis barleeaanum*, *Pullenia bulloides*, *Quinqueloculina seminulum*, *Stainforthia fusiformis*, and *Uvigerina* spp.; Gullmar Fjord species included *Bolivina skagerrakensis*, *Bolivinellina pseudopunctata*, *Bulimina marginata*, *C. laevigata*, *Elphidium excavatum*, *G. turgida*, *H. balthica*, *Nonionella turgida*, *Nonionellina labradorica*, *Pyrgo williamsoni*, *Q. seminulum*, and *S. fusiformis*; Charleston Bump species included *Bulimina aculeata*, *Bulimina marginata*, *Bulimina mexicana*, *Cibicoides* spp., *Lenticulina* sp., *Melonis barleeaanum*, and *Uvigerina* spp.; Bahamas species included *Bulimina aculeata*, *Bulimina marginata*, *Bulimina mexicana*, *C. laevigata*, *Cibicides lobatulus*, *Cibicoides* spp., *Globobulimina* sp., *Lenticulina* sp., *Melonis barleeaanum*, and *Uvigerina* spp. As noted, three culture chambers contained “monocultures” of the *Bulimina aculeata*/*B. marginata* complex. These two species were not separated, as juveniles of these two species were indistinguishable, and even the adult populations exhibited a spectrum of morphologies – some specimens appeared as typical *B. marginata* or *B. aculeata* while others exhibited traits of both species. Thus, we refer to our results on these species from the Charleston Bump and Great Bahama Bank to be from the *Bulimina aculeata*/*B. marginata* species complex.

The experiment was initiated on 19 December 2006, and was terminated between 15 and 23 May 2007. Five culture chambers were placed in each of three refrigerators (4, 7, 14 °C) and, in addition, two were placed at room temperature (~21 °C). Temperatures were monitored using Onset Tidbit T loggers. The culture chambers were individually plumbed, each with two outlets and two inlets at the top and at the bottom, to avoid geochemical gradients in the culture chambers, following Hintz et al. (2004). All culture chambers were connected to one single reservoir (200 L) of filtered surface seawater from the Gulf Stream, which was diluted with 8.4 L of MilliQ water to achieve a salinity of 35; this dilution also lowered the alkalinity from 2390 µeq/kg to 2258 µeq/kg. The water from the large reservoir was pumped to a header tank and both tanks were continuously bubbled with air from the building air intake. The air was first bubbled through a carboy of distilled water to increase its relative humidity. From the header tank, the water flowed to culture chambers by gravity; flow rates averaged 4 ml/min. The residence time of water in culture chambers was approximately 2 min.

Every 10 days, flow was stopped and a mixture of *D. tertiolecta* and *I. galbana* was introduced into each culture chamber to allow the foraminifera to feed. After an hour, the flow was reinstated after the algae settled to the silica surface. After experiment termination, the material from each culture chamber was rinsed over a 63-µm sieve and sorted into ju-

veniles (63–200 µm), young adults (200–300 µm) and adults (> 300 µm). Non-fluorescent calcite, which formed during the experiment as new chambers or offspring, was identified using epifluorescence microscopy as noted above.

Sampling for salinity, alkalinity, dissolved inorganic carbon (DIC), and $\delta^{18}\text{O}_w$ from the reservoir tank occurred approximately every third week. Salinity samples were measured by the WHOI Hydrographic Analysis Facility using an Autosal salinometer. The alkalinity and DIC data were analyzed by closed-vessel titration of large-volume (~100 ml) samples using an automated titration system (Bradshaw et al., 1981; Brewer et al., 1986), and the alkalinity and DIC concentrations were determined using a nonlinear curve fitting approach (Department of Energy, 1994), and standardized using certified reference materials obtained from Dr. A. Dickson (Scripps Institution of Oceanography). The standard deviation of replicate analyses of culture water was 0.4 µeq/kg for alkalinity and 1.3 µmol/kg for DIC ($n=6$ pairs). The carbonate ion concentration $[\text{CO}_3^{2-}]$ and the pH (total scale, “pH(t)”) were calculated from the alkalinity and DIC data using the “CO2SYS” program (Lewis and Wallace, 1998), with the carbonate dissociation constants of Roy et al. (1993), the calcite solubility of Mucci (1983), and the assumption that the boron/salinity ratio of the culture system water was equal to the seawater value.

The $\delta^{18}\text{O}$ of water was analyzed in the laboratory of Dr. D. Schrag (Harvard Univ.) using a VG Optima mass spectrometer with a VG Isoprep 18 automated shaker/equilibrater (Schrag et al., 2002). The standard deviation of replicate $\delta^{18}\text{O}$ analyses was 0.04‰ ($n=6$); $\delta^{18}\text{O}_w$ values are reported relative to Standard Mean Ocean Water (SMOW).

2.2 Stable oxygen isotopes of foraminiferal calcite

The stable oxygen isotopic composition of foraminiferal calcite was determined using a Kiel III Carbonate Device connected to a Finnigan MAT 253 mass spectrometer. The instrument was calibrated via NBS-19 and NBS-18 standards, with a long-term precision of replicate NBS-19 and in-house standards of <0.08‰ (Lynch-Stieglitz et al., 2009). Procedures and precision for this instrument can be found at: <http://www.whoi.edu/paleo/mass-spec/>. Depending on available calcite mass, samples consisted of pooled individuals ranging from 3 to 80 individuals or portions of individuals (see below). Foraminiferal stable isotopic data is expressed relative to VPDB. The paleotemperature equation

$$(T = 16.5 - 4.80 \cdot (\delta^{18}\text{O}_{c, \text{VPDB}} - (\delta^{18}\text{O}_{w, \text{SMOW}} - 0.27)))$$

was used to calculate $\delta^{18}\text{O}$ equilibrium values ($\delta^{18}\text{O}_{\text{Oeq}}$), the $\delta^{18}\text{O}$ values of calcite ($\delta^{18}\text{O}_{c, \text{VPDB}}$) in equilibrium with water ($\delta^{18}\text{O}_{w, \text{SMOW}}$) at the given temperature (Bemis et al., 1998).

2.3 Microdissection using laser ablation

In cases where an inoculate specimen added chambers during the experiment, the pre-existing (fluorescent) calcite was removed from the non-fluorescent calcite formed during the experiment using laser ablation microdissection (Hintz et al., 2006b). A conservative approach was used when microdissecting. We preferred to lose some of the newly added calcite rather than risk inclusion of pre-existing calcite in the subsequent analyses.

2.4 Mg/Ca analysis

Benthic foraminifera from deep-sea sediments are typically cleaned before solution ICP-MS Mg/Ca analysis, and several protocols (e.g., Martin and Lea, 2002) have been designed to remove clay and adhering contaminants that may be high in Mg relative to foraminiferal calcite, as well as high-Mg phases of calcite known to occur in some planktonic foraminifera (e.g., Brown and Elderfield, 1996; Dekens et al., 2002). The specimens in this study were not cleaned because the cultured foraminifera were lightly calcified and fragile, and rigorous chemical cleaning would likely have precluded analysis at 4 °C and reduced the number of analyses possible at 7, 14 and 21 °C. This approach seemed justified because the culture substrate (HPLC-grade silica) was clean, minimizing the risk of contamination.

Specimens chosen for Mg/Ca analysis were affixed to trimmed, gridded micropaleontology slides with gum tragacanth, and individually photographed under both reflected light and epifluorescence microscopy before analysis. Several mm-sized crystals of the OKA carbonatite standard (Bice et al., 2005) were also affixed to each slide. Mg/Ca analyses were made using the Thermo Element HR-ICP-MS equipped with a New Wave UP213 laser ablation sampling device. Raster patterns were defined in the NewWave software, using each specimen's micrographs to guide selection of a suitable sampling region. Raster paths were typically spaced 30 µm from one another but on several specimens with few experimentally precipitated chambers the paths were condensed to 20 µm spacing. Scan speed was 5 µm/s, repetition rate 10 Hz, scan depth 0 µm, spot size 40 µm and laser power 50%. Ablated material was removed from the chamber by helium carrier gas (0.42–0.5 L/minute) which was combined with argon (0.8 L/min) to form the final sample gas. Mass spectrometer settings closely followed those outlined in Hathorne et al. (2003). Isotope masses monitored were ²⁵Mg, ⁴³Ca, ⁴⁸Ca, ⁸⁶Sr, and ⁸⁸Sr, with each analysis consisting of 70 to 90 cycles through these five masses.

Blanks (run without laser input) and the OKA carbonatite standard were analyzed after every 3–6 samples, and ²⁵Mg and ⁴³Ca curves were constructed for blanks and standards analyzed over the course of each day's analyses. These blank values were subtracted from both samples and standards, and the sample ²⁵Mg/⁴³Ca ratio was normalized to an

OKA Mg/Ca of 4.55 mmol/mol, as determined by (Bice et al., 2005). In some cases, Mg and Ca intensities varied significantly over the course of an analysis. When Mg and Ca intensities peaked simultaneously this was interpreted as the result of rapid introduction of calcite to the mass spectrometer, perhaps when a larger-than-typical amount of calcite was ablated. Other, less regular fluctuations in signal intensity, including the anticorrelation of Mg and Ca, may reflect instrument tuning problems, though we cannot rule out the possibility of extremely heterogeneous material. Only those analyses in which the individual peak intensities remained stable over a minimum of 25 contiguous cycles were considered acceptable; analyses not meeting this criterion were rejected. Mg/Ca error for each data point was determined using a standard error propagation formula (Weiss, 2004). Analyses of the OKA carbonatite standard provide one estimate of the reproducibility of LA-ICP-MS Mg/Ca measurements: the relative standard deviation for 12 OKA measurements made over two months was 1.7%. However, no direct test of the reproducibility of LA-ICP-MS Mg/Ca measurements of foraminiferal calcite was possible because specimens are likely to exhibit intratest and intertest heterogeneity (Pena et al., 2008).

3 Results

3.1 Water chemistry

The cytoplasm of all specimens born in culture was green at the end of the experiment, indicating active feeding at that time. We assume that most calcification by these specimens, and by the adults as well, occurred in the final months of the experiment, and focus on water chemistry between February and May 2007. The salinity increased from 35.0 to ~35.8 during the entire experiment (Fig. 2a), and $\delta^{18}\text{O}_w$ increased from 0.7‰ to 0.95‰ (Fig. 2b). The average $\delta^{18}\text{O}_w$ value between February and May was 0.9‰ (range 0.1‰) and the average temperatures for the same period were 3.8 °C (range ~1 °C), 7.0 °C (range 0.5–1 °C), 14.1 °C (range 0.5–1 °C), 21.0 °C (range 1–2 °C) (Fig. 2c), abbreviated to 4, 17, 14, and 21 °C. The coldest and warmest treatments showed the most temperature variation. Temperature did not systematically increase or decrease over the experiment, although long-term trends were noted in the 21 °C treatment, which was not thermostatted.

Alkalinity increased by 0.7% during the course of the experiment, from 2258 µeq kg⁻¹ to 2273 µeq kg⁻¹ (Fig. 3a, Table 2). This increase is smaller than would be predicted from the observed salinity increase, suggesting a modest consumption of alkalinity, either due to the release of metabolites, or to leaching of trace acidity from the system components (tubing, culture chambers, etc.) during the experiment. Dissolved inorganic carbon (DIC) showed a similar increase through most of the experiment, but decreased in

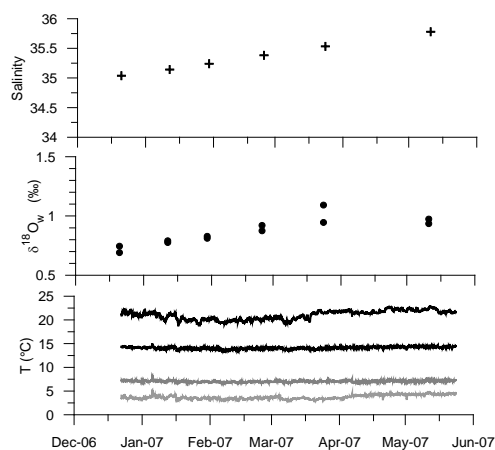


Fig. 2. Salinity, $\delta^{18}\text{O}_w$, and temperature measured during the experiment. Two water samples from the reservoir tank were taken for salinity and $\delta^{18}\text{O}_w$ at each sampling occasion, temperature was measured continuously with data loggers.

Table 2. Water chemistry measured during the course of the experiment together with calculated values of pH(total scale), CO_3^{2-} and Ω . The average measured salinity, alkalinity, and DIC of the 200L reservoir (35.35 (SD 0.26); 2264.0 $\mu\text{eq/kg}$ (SD 6.6), and 2001.0 $\mu\text{mol/kg}$ (SD 6.8)) were used for calculations at all four temperatures, the standard deviations are presented within parenthesis, shorten to SD.

Temperature (°C)	Calc. pH (total scale)	Calc. $[\text{CO}_3^{2-}]$ ($\mu\text{mol/kg}$)	Ω
3.8	8.304	182.5	4.30
7.0	8.248	182.7	4.35
14.1	8.129	187.5	4.46
21.0	8.018	191.5	4.57

April and May 2007 (Fig. 3a). Because alkalinity and DIC covaried, the carbonate ion concentration $[\text{CO}_3^{2-}]$ remained relatively constant at values between 188 and 191 $\mu\text{mol/kg}$, increasing to 202 $\mu\text{mol/kg}$ in the final sample (Fig. 3b). The pH(t) showed a similar trend, ranging between 8.00 and 8.03 (Fig. 3b).

3.2 Calcification at different temperatures

Bulimina aculeata and *B. marginata* were the most successful taxa in the experiment, in terms of calcification, so we focus on these two species. In addition, *Cassidulina laevigata* also reproduced at 7 °C. The reproductive yield of *C. laevigata* was sparse, and the mass produced was insufficient for proxy analyses. Specimens of *Hyalinea balthica* and *Melonis barleeanum*, originally from the Skagerrak, also added a few new chambers at all temperatures except at 21 °C. The

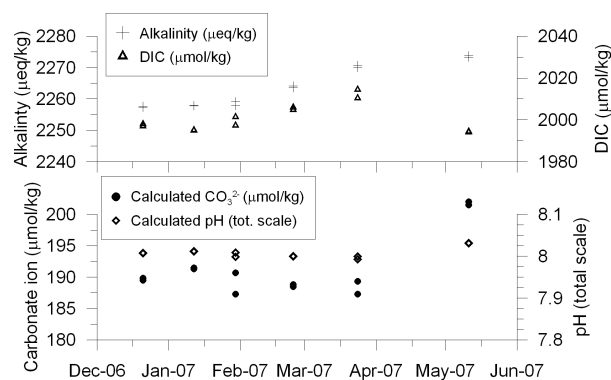


Fig. 3. Water chemistry data during the course of the experiment. Alkalinity and DIC were analyzed from water samples from the reservoir tank, and used to calculate $[\text{CO}_3^{2-}]$ and pH(t).

specimens originating from the Gullmar Fjord grew little and did not reproduce at any of the temperatures; we omit further discussion of the fjord material. A few specimens of *Rosalina* sp. occurred in all culture chambers except those at room temperature, despite not being observed in the initial inoculate.

At the lowest temperature (4 °C), several specimens of *B. aculeata/B. marginata* added one to three chambers to their tests, but little growth occurred in the remaining 4 °C specimens, and no reproduction took place at 4 °C (Table 3). At 7 °C, *B. marginata* from the Skagerrak reproduced abundantly, yielding approximately 90 new specimens, which had reached the 63 to 200 μm size fraction (the smallest fraction collected) by the end of the experiment (Table 3). Considerable growth of *B. aculeata/B. marginata* occurred in the three remaining 7 °C culture chambers, enabling microdissection of new (experimental) calcite from many specimens (Table 3). Reproduction also occurred at 14 °C, producing hundreds of offspring that had reached the 63–200 μm size fraction by the end of the experiment (Table 3). Some specimens of the *B. aculeata/B. marginata* complex also reproduced at 14 °C, and a large proportion of those offspring grew to the >300 μm size fraction (Table 3). It is likely that more than one reproduction event occurred at this temperature because there were specimens ranging from juveniles to adult that were all born in culture (Table 3). Dozens of inoculate specimens displayed substantial new growth, adding at least three chambers and increasing in test volume by up to a factor of 5. In the two culture chambers at room temperature (21 °C), reproduction did not occur; those specimens that grew added just one or two new chambers (Table 3).

Table 3. Number of inoculated specimens from the different source regions together with the results from the culturing experiment, organized according to temperature and size classes. “Monoculture” refers to monocultures of *Bulimina aculeata/marginata*, originating from the Charleston Bump and near the Great Bahama Bank. Juveniles, young adults, and adults were born in culture; “new growth” is material precipitated during the experiment.

Culture chamber	Geographical source area	T (°C) (experimental)	No. of inoculated <i>B. aculeata/B. marginata</i>	Juveniles (63–200 μm)	Young adults (200–300 μm)	Adults (>300 μm)	New growth (no. micro-dissected specimens)
13	Skagerrak	3.8	19	—	—	—	6
14	Gullmar Fjord	3.8	12	—	—	—	—
15	C. Bump	3.8	50	—	—	—	2
16	Monoculture	3.8	~ 60	—	—	—	6
17	Bahamas	3.8	64	—	—	—	—
7	Skagerrak	7.0	20	70	17	5	6
8	Gullmar Fjord	7.0	12	—	—	—	—
9	C. Bump	7.0	50	—	—	—	19
10	Monoculture	7.0	~ 60	—	—	—	24
11	Bahamas	7.0	62	—	—	—	48
1	Skagerrak	14.1	20	~ 100	38	9	—
2	Gullmar Fjord	14.1	10	—	—	—	—
3	C. Bump	14.1	50	8	16	24	23
4	Monoculture	14.1	~ 60	2	11	42	11
5	Bahamas	14.1	62	—	—	—	47
19	Skagerrak	21.0	0	—	—	—	—
20	Monoculture	21.0	30	—	—	—	9

3.3 Stable oxygen isotopes

The amount and type of test material available for *B. aculeata/B. marginata* stable isotope analyses (microdissected new growth vs. offspring born in culture) varied between the different temperatures (Table 4). In general, larger specimens (>300 μm) at 7 °C were closer to $\delta^{18}\text{O}_{\text{eq}}$ than smaller specimens (Fig. 4a). At 14 °C the oxygen isotope data from all size classes were similar, and slightly higher than the predicted $\delta^{18}\text{O}_{\text{eq}}$, except for the 14 °C juveniles which had lower $\delta^{18}\text{O}$ values (Fig. 4b). For comparison with the cultured specimens, stable isotopic analyses were also performed on pre-existing calcite from samples originating from the Skagerrak, Charleston Bump, and Great Bahamas Bank (Table 4); the $\delta^{18}\text{O}$ values from the pre-existing calcite varied depending on source region where the largest scatter came from the monocultures of *B. aculeata/marginata* complex.

3.4 Mg/Ca ratios

Mg/Ca analyses were performed on microdissected, new growth from all four temperatures, and on young adults and adult specimens born in culture from the 14 °C treatment. The foraminiferal Mg/Ca values ranged from 3.4 to 22.7 mmol/mol (Table 5, Fig. 5). No significant difference in Mg/Ca was observed between specimens from different culture chambers maintained at a single temperature (e.g., culture chambers 3 and 10 at 7 °C; culture chambers 15 and 16 at 4 °C), and the Mg/Ca of specimens born in culture at 14 °C did not differ significantly from that of chambers added during the course of the experiment at this temperature (Fig. 5).

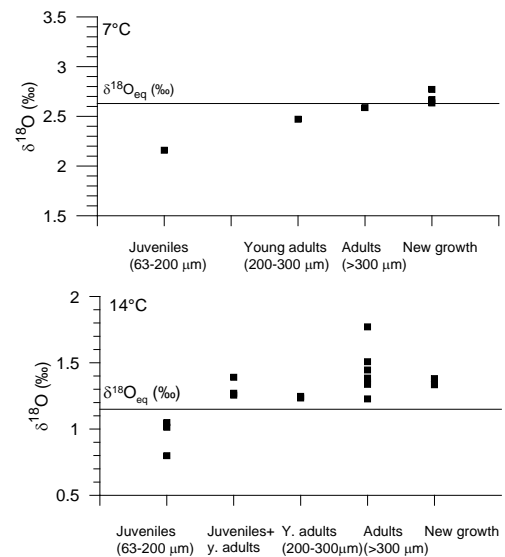


Fig. 4. The uppermost panel (a) shows $\delta^{18}\text{O}$ values at 7 °C and the lower panel (b) for 14 °C for the different size classes, together with the equilibrium calcite line for each temperature.

4 Discussion

4.1 Growth and reproduction with respect to temperature

Bulimina aculeata and *B. marginata* occur widely, ranging latitudinally as well as by water depth, occurring from deltas to the deep sea (Murray, 2006). In the South Atlantic

Table 4. Stable oxygen isotopic data for the cultured foraminifera presented for each temperature and size class, together with isotope data for pre-existing calcite. The standard deviations refer the analytical precision of the mass spectrometer. “Monoculture” refers to monocultures of *Bulimina aculeata/marginata*, originating from the Charleston Bump and near the Great Bahama Bank. N refers to number of specimens or microdissected parts of specimens analyzed. * = small samples with lower analytical precision.

Culture chamber	Source area	T (°C)	Material	$\delta^{18}\text{O}$ mean (‰)	$\delta^{18}\text{O}$ SD (‰)	N
13+15+16	Skagerrak+C. Bump+Monoculture	3.8	New Growth	1.27	0.06	13
7	Skagerrak	7.0	Juveniles	2.16	0.03	74
7	Skagerrak	7.0	Young adults	2.47	0.04	15
7	Skagerrak	7.0	Adults	2.59	0.03	5
9	C. Bump	7.0	New growth	2.67	0.04	6
10	Monoculture	7.0	New growth	2.63	0.02	10
11	Bahamas	7.0	New Growth	2.77	0.03	10
1	Skagerrak	14.1	Juveniles	0.80	0.04	80
1	Skagerrak	14.1	Juveniles	1.01	0.04	42
1	Skagerrak	14.1	Young adults	1.25	0.02	20
1	Skagerrak	14.1	Young adults	1.23	0.01	24
1	Skagerrak	14.1	Adults	1.23	0.04	5
1	Skagerrak	14.1	Adults	1.77	0.05	4
3	C. Bump	14.1	Juv.+ young adults	1.27	0.03	13
3	C. Bump	14.1	Juv.+ young adults	1.26	0.03	14
3	C. Bump	14.1	Adults	1.34	0.03	5
3	C. Bump	14.1	Adults	1.44	0.04	5
4	Monoculture	14.1	Juv.+ young adults	1.39	0.04	13
4	Monoculture	14.1	Adults	1.35	0.02	8
4	Monoculture	14.1	Adults	1.38	0.02	8
4	Monoculture	14.1	Adults	1.51	0.04	8
5	Bahamas	14.1	New growth	1.33	0.02	6
5	Bahamas	14.1	New growth	1.38	0.05	6
20	Monoculture	21.0	New growth	-0.20*	0.17	
3	C. Bump	NA	Pre-existing	-0.79	0.04	8
4	Monoculture	NA	Pre-existing	0.14	0.05	8
5	Bahamas	NA	Pre-existing	-0.64	0.02	20
9	C. Bump	NA	Pre-existing	-0.11	0.05	10
10	Monoculture	NA	Pre-existing	0.13	0.04	13
11	Bahamas	NA	Pre-existing	0.16	0.02	16
13	Skagerrak	NA	Pre-existing	2.69	0.05	5
15	C. Bump	NA	Pre-existing	-1.55*	0.05	3
20	Monoculture	NA	Pre-existing	-0.76	0.09	9

Ocean, *B. aculeata* is present at sites with temperatures as low as 0.5 °C (Mackensen et al., 1993) demonstrating that this species can grow and reproduce below 4 °C. Here, however, the cultured specimens grew at 4 °C, but they did not reproduce. The cultured specimens may not have been adapted to this lowest temperature; the temperatures at the coring sites ranged from ~7 °C (Skagerrak) to ~8–10 °C on the Charleston Bump and the Great Bahama Bank (Bernhard et al., 2006, SMHI database SHARK). At the lowest culture temperature, growth is likely to be slow, so a longer experiment may have been required to produce similar mass as at higher experimental temperatures. The specimens from the Charleston Bump and the Bahamas grew at 7 °C, but only

the *Bulimina marginata* specimens from the Skagerrak reproduced at this temperature. This temperature is similar to the average temperature at the sampling site in NE Skagerrak, and is slightly warmer than the reported lower temperature limit for *B. marginata* (Husum and Hald, 2004). The highest yield reproduction events took place at 14 °C, and were observed in specimens from all collection sites. This temperature is considerably higher than the temperature at any of the sampling sites. However, we note that both species (*B. aculeata* and *B. marginata*) have been reported from areas with relatively high temperatures; *B. marginata* for instance from the South Brazilian Shelf (13–20 °C) (Eichler et al., 2008) and the Red Sea (Edelman-Furstenberg et al.,

Table 5. Mg/Ca data for the calcite precipitated during the experiment presented for each temperature and analyzed material (new growth; young adults and adults) together with specimen length and length to LA raster.

Culture chamber	<i>T</i> (°C)	Material	Total length of specimen (μm)	Length to analyzed calcite (μm)	Mg/Ca (mmol/mol) uncertainty (%)	Mg/Ca
15	3.8	New growth	238	145	10.91	21
15	3.8	New growth	374	274	18.43	7
16	3.8	New growth	188	152	22.70	23
10	7.0	New growth	553	249	5.92	5
10	7.0	New growth	478	297	4.83	7
10	7.0	New growth	432	307	4.22	7
9	7.0	New growth	630	400	4.50	7
9	7.0	New growth	425	354	3.92	7
10	7.0	New growth	483	314	2.97	7
10	7.0	New growth	414	284	3.97	7
9	7.0	New growth	395	321	6.35	9
9	7.0	New growth	527	390	6.13	7
9	7.0	New growth	505	341	6.04	7
9	7.0	New growth	505	343	6.15	7
9	7.0	New growth	434	345	9.78	7
9	7.0	New growth	472	343	6.19	9
9	7.0	New growth	528	379	3.35	5
3	14.1	Young adult	240	134	5.18	8
3	14.1	Young adult	265	111	6.22	8
3	14.1	Adult	331	220	6.97	8
3	14.1	Adult	398	275	7.46	8
3	14.1	Adult	352	289	6.69	8
3	14.1	New growth	551	363	4.55	8
3	14.1	New growth	529	405	10.17	8
3	14.1	New growth	450	302	10.07	8
3	14.1	New growth	465	324	3.92	8
3	14.1	New growth	465	305	6.15	8
20	21.0	New growth	401	304	9.48	5
20	21.0	New growth	376	255	8.25	6
20	21.0	New growth	410	330	7.89	6

2001), and *B. aculeata* from the Ebro Delta (14–19 °C) (Murray, 2006; Cartes et al., 2007).

We do not know whether successful reproduction in culture implies that the slightly warmer temperature was more favorable for the foraminifera, or whether reproduction was a stress response to the elevated temperature. The absence of reproduction at higher (21 °C) and lower (4 °C) temperatures suggests that temperature stress does not consistently promote reproduction. These results – elevated reproduction at intermediate temperatures – are consistent with the observations of Barras et al. (2009).

4.2 Ontogenetic trends in isotopic values

A substantial fraction of the observed oxygen isotopic variability is related to test size, suggesting age-related (ontogenetic) effects on test chemistry (Fig. 4). Several studies have suggested that younger (smaller) individuals precipitate CaCO₃ with lower δ¹⁸O values than older (larger) specimens

(e.g., Dunbar and Wefer, 1984; Schmiiedl et al., 2004; McCorkle et al., 2008), though this pattern does not always exist (e.g., Wefer and Berger, 1991; Corliss et al., 2002). A size-linked (ontogenetic) trend is seen in the 7 °C δ¹⁸O data, with the most pronounced depletions in the smallest specimens (Fig. 4a). This pattern is less clear in the 14 °C data, where all groups >200 μm show similar δ¹⁸O values (Fig. 4b). Ontogenetic isotopic depletions were most pronounced in the smallest size fractions analyzed by McCorkle et al. (2008), who suggested that adult specimens approach an asymptotic isotopic composition. Indeed, our data suggest a tendency for older (larger) specimens to approach isotopic equilibrium.

4.3 Oxygen isotopic composition as a function of temperature

Variability in foraminiferal δ¹⁸O_c, including the impact of ontogenetic effects, can be assessed by considering the offsets (Δδ¹⁸O values) between the observed foraminiferal

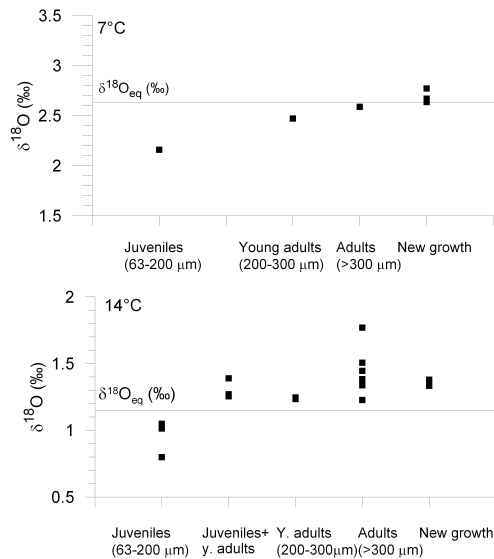


Fig. 5. Mg/Ca (mmol/mol) over the experimental temperature gradient, presented by sample type (new growth and size classes). For 7 and 14 °C, data is presented in two columns to allow visualization of result from different samples types, see legend for details.

$\delta^{18}O_c$ and the predicted foraminiferal $\delta^{18}O_{eq}$ for each experimental temperature (Fig. 6). These $\Delta\delta^{18}O$ values were calculated using the paleotemperature equation from Bemis et al. (1998). Where there are sufficient data (7 °C and 14 °C), similar $\delta^{18}O_c$ values (Table 4) and $\Delta\delta^{18}O$ values (Figure 6) are seen in both new growth and born-in-culture specimens. The range in $\Delta\delta^{18}O$ for new growth and adult specimens at the three warmest temperatures (roughly 0.6) was only slightly larger than the range of $\Delta\delta^{18}O$ values previously observed in single-temperature culturing experiments, which ranged between 0.2 and 0.5‰ (McCorkle et al., 2008). The observed $\Delta\delta^{18}O$ range is also similar to that reported for field samples of *B. aculeata*, (McCorkle et al., 1997; Mackensen et al., 2000).

The foraminiferal $\delta^{18}O_c$ values decreased with increasing temperature from 7 °C to 21 °C (Fig. 7). However, the single, unreplicated value at 4 °C (0.62‰) is roughly 2‰ lower than we would expect from the paleotemperature equation estimates discussed below, and instead is similar to the $\delta^{18}O_c - \delta^{18}O_w$ values of the 14 °C specimens (Fig. 7). This 4 °C isotopic analysis used microdissected, new growth calcite from multiple specimens, raising the possibility that the sample inadvertently included some pre-existing calcite, precipitated at a higher temperature in the field. We do not think that this occurred, but while we have no reason simply to reject the data point at 4 °C, we will not use it in the following assessment of the temperature dependence of *Bulimina* $\delta^{18}O$.

The $\delta^{18}O_c - \delta^{18}O_w$ values provide a culture-based assessment of published oxygen isotope paleotemperature equa-

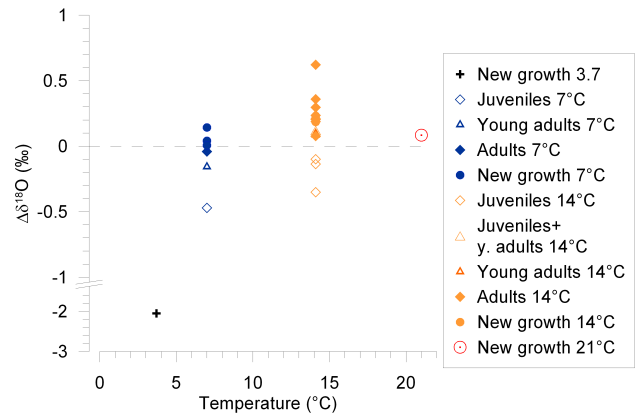


Fig. 6. The difference between $\delta^{18}O_c$ and the $\delta^{18}O_{(eq)}$ vs experimental temperature. $\delta^{18}O_{(eq)}$ is indicated with a dotted line and calculated using the Bemis et al. (1998) paleotemperature equation, together with a $\delta^{18}O_w$ of 0.92 (average between FeB. and May). All calcite was precipitated during the experiment; the symbols refer to the different material, see legend for details.

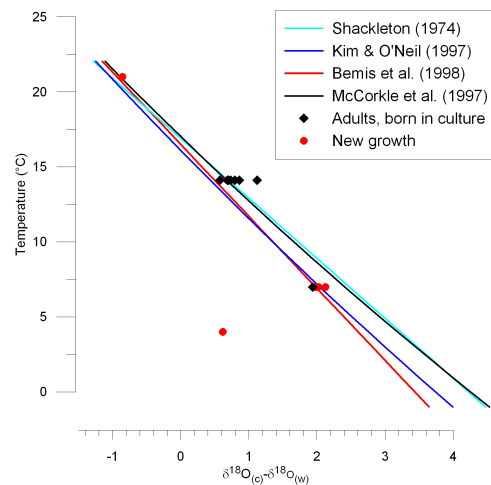


Fig. 7. Temperature vs. $\delta^{18}O_c - \delta^{18}O_w$, together with the paleotemperature equations from Shackleton (1974), Kim and O'Neil (1997), McCorkle et al. (1997), and Bemis et al. (1998). The symbols represent calcite precipitated during the experiment.

tions (Fig. 7) (Shackleton, 1974; Kim and O'Neil, 1997; McCorkle et al., 1997; Bemis et al., 1998). Although the 14 °C culture data are most consistent with the “warmer” paleotemperature equations (Shackleton, 1974; McCorkle et al., 1997), the best overall agreement, over the 21 °C to 7 °C temperature range, is with the paleotemperature equation of Bemis et al. (1998) (Fig. 7).

A previous culture study reported small depletions from predicted equilibrium calcite $\delta^{18}O$ values (McCorkle et al., 2008) and proposed that these isotopic offsets could reflect the elevated carbonate ion concentration of the culture system, relative to typical bottom-water values, following the

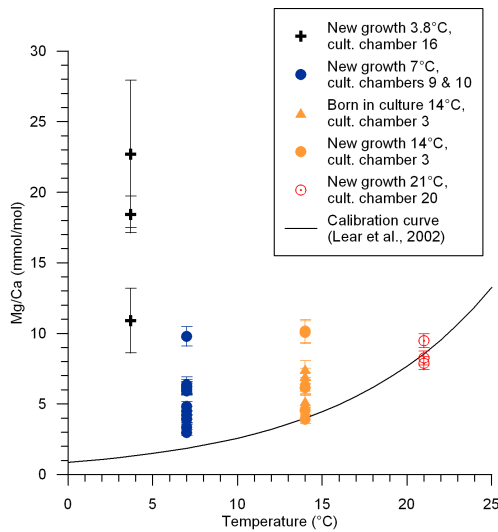


Fig. 8. Mg/Ca vs Temperature including *Cibicidoides* core top calibration of Lear et al. (2002), $\text{Mg/Ca} = 0.867 \pm 0.49 \exp(0.109 + 0.007 \cdot \text{BWT})$, where BWT is bottom water temperature).

results of Spero and co-workers (Lea and Spero, 1992; Spero et al., 1997; Bijma et al., 1999). However, the Bemis et al. (1998) paleotemperature expression, which provides the best match to the multiple temperature results of this study, would have been in better agreement with the $\delta^{18}\text{O}$ data of McCorkle et al. (2008), obviating the need to invoke a carbonate ion effect. The higher-than-predicted $\delta^{18}\text{O}$ values in the 14 °C cultures cannot be explained by elevated carbonate ion concentrations, which are expected to cause depleted isotopic values (and which were, in any case, present at all four temperatures). At present, we have no proposed mechanism for the 14 °C offset.

4.4 Mg/Ca ratios with respect to temperature

The foraminiferal Mg/Ca values from the 21 °C treatment vary by roughly $\pm 10\%$ about a mean of 8.5 mmol/mol, but the Mg/Ca values from the other three temperatures show much more scatter, varying by factors of 2 to 3. In addition to this variability, the absolute values of the foraminiferal Mg/Ca ratios in the 4, 7, and 14 °C treatments are much higher than the values predicted by studies of core-top field specimens. To illustrate this offset, we plot our culture data with the benthic foraminiferal (*Cibicidoides*) Mg/Ca temperature calibration curve of Lear et al. (2002) (Fig. 8). The Mg/Ca values from the 21 °C cultures span the Lear et al. calibration curve, but the remainder of our Mg/Ca data are substantially higher than predicted by the Lear et al. (2002) relationship, and other published calibrations (Martin et al., 2002; Healey et al., 2008). Anand and Elderfield (2005) and Sadekov et al. (2005, 2008) observed high intra-test variability in the Mg/Ca of planktonic foraminifera, and inferred that factors other than calcification temperature can influence test

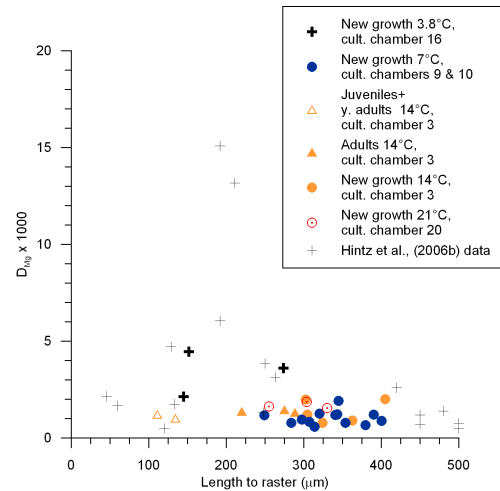


Fig. 9. Mg distribution coefficients plotted versus length from the proloculus to LA raster, presented together with the data from Hintz et al. (2006a) study of ontogenetic effects on Mg/Ca of *B. aculeata*. D_{Mg} for the cultured foraminifera from the present study was calculated assuming a sea water Mg/Ca ratio of 5.109.

Mg/Ca. However, while deep-water Mg/Ca paleothermometry continues to be a topic of active study and debate, none of the field-based data sets report low-temperature values approaching those we observed (Lear et al., 2002; Martin et al., 2002; Marchitto et al., 2007; Healey et al., 2008).

We do not believe that the high Mg/Ca values reflect species-dependent variation in Mg incorporation. We know of two sets of Mg/Ca analyses of field collected *Bulimina*, including ~ 4.5 °C deep-sea data from Lear et al. (2002), and shallow water data (~ 7 °C) from the Skagerrak and Gullmar Fjord (Groeneveld and Filipsson, personal communication). These *Bulimina* field data agree well with the Lear et al. (2002) *Cibicidoides* calibration curve, and include no values higher than ~ 2.5 mmol/mol.

Dissard et al. (2010) reported no difference between the Mg/Ca values of the shallow-water benthic foraminifera *Ammonia tepida* cultured at 10 °C and at 20 °C. However, strongly elevated *Bulimina* Mg/Ca values were observed in a previous culturing study (Hintz et al., 2006b). Single-individual whole-specimen Mg/Ca values, cultured at 7 °C and measured by solution ICP-MS, reached values equal to the highest LA-ICP-MS values observed in this study, and included several specimens with Mg/Ca values above 5 mmol/mol. In Hintz et al. (2006b), elemental analyses of laser microdissected chambers revealed that chambers located between ~ 150 – 225 μm from the test apex – similar in size to our study's 4 °C foraminifera – had the highest Mg contents (Fig. 9). Ontogenetic effects, and their underlying variations in calcification mechanisms, may explain the elevated Mg/Ca values in our study. However until these trends have been observed again over a temperature range,

and cleaning and analytical artifacts including the possible effect of the Mg content in the algal chlorophyll have been further tested and ruled out, we are unwilling to take these first multiple temperature culture-based Mg/Ca data at face value.

5 Conclusions

We cultured benthic foraminifera at 4, 7, 14, and 21 °C, and observed reproduction and copious calcification of *Bulimina aculeata/marginata* at temperatures close to or slightly higher than in situ temperatures (7 and 14 °C treatments), with no reproduction and less calcification at either substantially lower (4 °C) or higher (21 °C) temperatures. The absence of reproduction at higher and lower temperatures suggests that temperature stress does not consistently promote reproduction; instead there appears to be a temperature window where environmental conditions are most beneficial for these species' reproduction and growth.

Observed ontogenetic variations in foraminiferal stable oxygen isotope values are consistent with patterns observed in culturing studies by McCorkle et al. (2008) and Barras et al. (2010). Further, the observed $\delta^{18}\text{O}$ data are more consistent with the paleotemperature equation proposed by Bemis et al. (1998) than with those proposed by e.g., Shackleton (1974), and McCorkle et al. (1997). In contrast, the high observed foraminiferal Mg/Ca values in this study are not consistent with published benthic foraminiferal Mg/Ca: temperature relationships. This discrepancy may reflect ontogenetic variations in foraminiferal elemental compositions, but at present we cannot rule out sample cleaning or analytical explanations for these high values.

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