Biogeosciences, 7, 1349–1356, 2010
www.biogeosciences.net/7/1349/2010/
doi:10.5194/bg-7-1349-2010
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Biogeosciences

Calibration of δ^{18}O of cultured benthic foraminiferal calcite as a function of temperature

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Received: 16 December 2009 – Published in Biogeosciences Discuss.: 18 January 2010
Revised: 13 April 2010 – Accepted: 20 April 2010 – Published: 29 April 2010

Abstract. The geochemical composition of deep-sea benthic foraminiferal calcite is widely used to reconstruct sea floor paleoenvironments. The calibration of the applied proxy methods has until now been based on field observations in complex natural ecosystems where multiple factors are interfering. However, laboratory experiments with stable physico-chemical conditions appear to be the ideal way to evaluate the influence of a single parameter. In this paper, we present the oxygen isotopic composition of deep-sea benthic foraminiferal shells entirely calcified under controlled experimental conditions over a large temperature range (4 to 19 °C). The new laboratory protocols developed for this study allowed us to produce large quantities of shells in stable conditions, so that also the shell size effect could be investigated. It appears that when considering a narrow test size range, the curve describing the temperature dependency of δ^{18}O in *Bulimina marginata* is parallel to the thermodynamically determined curve observed in inorganically precipitated calcite (-0.22‰ °C^{-1}). This observation validates the use of δ^{18}O of this benthic species in paleoceanographical studies. Over the studied size range (50 to 300 µm), the effect of test size was 0.0014‰ µm^{-1}, confirming previous suggestions of a substantial test size effect on δ^{18}O of benthic foraminifera. This study opens new perspectives for future proxy calibrations in laboratory set-ups with deep-sea benthic foraminifera (e.g. quantification of the influence of the carbonate chemistry).

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1 Introduction

Stable oxygen isotopes of carbonate microfossils are one of the most widely used tools in paleoceanography. The temperature dependency of oxygen isotope fractionation has previously been quantified on the basis of inorganically precipitated calcite (Urey, 1947; McCrea, 1950; O'Neil et al., 1969; Kim and O'Neil, 1997), and has been verified for living organisms in field and/or laboratory cultures of corals (Reynaud-Vaganay et al., 1999), molluscs (Epstein et al., 1953) and planktonic foraminifera (Erez and Luz, 1983; Bouvier-Soumagnac and Duplessy, 1985; Bouvier-Soumagnac et al., 1986; Bemis et al., 1998). For benthic foraminifera, until now, all existing temperature calibrations are based on core top material. On the sea floor, not only temperature and the isotopic composition of the seawater influence the ^{18}O/^{16}O composition of foraminiferal calcite, but also other factors, such as the carbonate ion effect (Spero et al., 1997; Zeebe, 1999; Rathmann and Kunhert, 2008), vital effects (Duplessy et al., 1970) and diagenetic processes may strongly influence the δ^{18}O of carbonate microfossils. Since many of these factors co-vary in the natural environment, only culture experiments can precisely reveal the influence of a single parameter, such as temperature.

Several laboratory studies have been performed to study the oxygen isotopic fractionation in planktonic and shallow water benthic foraminifera (e.g. Erez and Luz, 1983; Bouvier-Soumagnac and Duplessy, 1986; Chandler et al., 1996; Spero and Lea, 1996; Spero et al., 1997; Bemis et al., 1998). However, experiments with deep-sea benthic foraminifera are very scarce (Wilson-Finelli et al., 1998; McCorkle et al., 2008; Filipsson et al., 2010). Actually, the growth of deep-sea benthic foraminifera takes much longer

than for planktonic foraminifera so that the experiments in stable conditions have to last for periods extending to several months. However, benthic foraminifera present the indisputable advantage that they can reproduce in the laboratory (Hintz et al., 2004; McCorkle et al., 2008; Barras et al., 2009; Filipsson et al., 2010). It is therefore possible to measure the isotopic composition of shells entirely calcified under controlled conditions.

In order to obtain the results presented in this paper, we developed new laboratory protocols to produce large quantities of *Bulimina marginata* shells under controlled and stable conditions and over a large range of temperatures (4–19 °C), making it possible to investigate the influence of temperature on the $\delta^{18}O$ of deep-sea benthic foraminiferal calcite. The large amount of foraminiferal shells produced allowed us also to investigate the effect of test size on isotopic fractionation.

2 Material and methods

2.1 Experimental protocols

For this study, adult specimens of *B. marginata* (non-symbiont-baring benthic species) sampled in the Bay of Biscay at 450 and 650 m depth, were used in different experiments to obtain reproduction and subsequent growth of the juveniles (detailed protocol and data on reproduction and growth rates of *B. marginata* in Barras et al., 2009). Before their introduction in the experiments, adult specimens were labelled using a calcein-tagging method (Bernhard et al., 2004) in order to distinguish specimens that totally calcified their shells in our controlled experiments (not fluorescent) from the adults initially added (partly fluorescent). Two different laboratory setups were used to obtain reproduction and growth of *B. marginata* under stable physico-chemical conditions: (1) a closed system (CS_I and CS_{II}), with 25 l microfiltrated (0.45 µm) natural seawater circulating through a reservoir and different experiment bottles, and (2) a Petri dish system (PD) where half of the seawater was renewed twice per week. Between 30 and 190 adult specimens of *B. marginata* were introduced in each experiment, which were regularly fed with fresh *Phaeodactylum tricornutum* diatoms. In all experiments, which lasted from 43 to 108 days, we obtained production and growth of juveniles of *Bulimina marginata*. Therefore, the isotopic composition of foraminiferal calcite was measured on tests of *Bulimina marginata* entirely calcified under controlled laboratory conditions (not fluorescent specimens).

Temperature was recorded inside the incubators (standard deviations range from 0.1 to 1.1 °C depending on the incubator). Culture water samples were collected every 3 to 7 days to verify the stability of salinity (35.8 ± 0.1), $\delta^{18}O_{seawater}$ (0.6 ± 0.1‰ vs. SMOW), pH and alkalinity, and the absence of significant evaporation (details in Table 1). The carbonate chemistry was stable, and similar in experiments CS_I and PD (7.94 ± 0.05 for pH, NBS-scale, and 2453 ± 34 µmol l^{-1} for alkalinity; Table 1). However, an episodic peak of high alkalinity and pH was recorded during the first week of the PD experiments, which is probably irrelevant for the geochemical composition of the newly formed shells, since *B. marginata* only reproduces after several weeks of incubation (Barras et al., 2009). For CS_{II}, a gradual decrease of pH by 0.3 units between the start and the end (average of 7.79 ± 0.09, NBS-scale) occurred in the six experiments, whereas alkalinity remained stable, and similar to the other systems (2523 ± 14 µmol l^{-1}) (Table 1). In the hypothetical case of linear growth of the shells during the experimental period, this gradual decrease of pH by 0.3 units could theoretically result in a positive $\delta^{18}O$ shift of about 0.15‰ of the newly formed foraminifera, due to the carbonate ion effect (Zeebe, 1999). However, benthic foraminifera do not have a uniform growth, chamber addition being faster during early ontogenetic stages (Bradshaw, 1957; Stouff et al., 1999; Barras et al., 2009).

2.2 Analytical procedures

Oxygen isotopic analyses were performed on 10 to 150 entire specimens of *B. marginata*. In order to study the ontogenetic effect on the $^{18}O/^{16}O$ ratios of the shells of deep-sea benthic foraminifera, specimens were separated into different size fractions (length measurements with microscale). Observation of the shells under the stereomicroscope showed that they were transparent with no mineral adhesives visible. Therefore specimens were only rinsed with deionised water before analysis. All tests were then roasted at 380 °C during 45 min to remove all organic matter. The $^{18}O/^{16}O$ ratio of foraminiferal calcite was measured with Isoprime and VG-Optima mass-spectrometers. Results are expressed as $\delta=((R_{sample}-R_{standard})/R_{standard})\cdot 1000$, where R is the $^{18}O/^{16}O$ isotopic ratio. The analytical precision of the $\delta^{18}O$ analyses is ± 0.05‰ relative to the VPDB (Vienna Pee Dee Belemnite) standard.

Seawater $\delta^{18}O$ ($\delta^{18}O_w$) was measured by equilibrating water samples with pure CO_2 which was subsequently analysed with a Finnigan Mass spectrometer. The analytical precision of the $\delta^{18}O$ analyses is ± 0.05‰ relative to the VSMOW (Vienna Standard Mean Ocean Water) standard.

In order to determine the relationship between temperature and $\delta^{18}O$ of *B. marginata* shells, we calculated least square regressions of the isotopic difference between foraminiferal shell and seawater ($\delta^{18}O_f - \delta^{18}O_w$) versus temperature. The $\delta^{18}O_w$ data were converted from VSMOW to VPDB by subtracting 0.27‰ (Hut, 1987). We applied linear regression to our data sets since this provided equally good fits as quadratic regression. The choice of linear or quadratic equations was discussed by Bemis et al. (1998). If we consider for example the paleotemperature equations of Kim and O'Neil (1969) and apply linear regression for the temperature range

Table 1. Physico-chemical parameters (temperature, salinity, pH and alkalinity) measured during PD, CSI and CSII experiments, total number of *B. marginata* shells entirely calcified under controlled conditions per experimental temperature, and δ^{18}O composition of the shells according to different size fractions.

Temperature (°C)	Salinity	pH (µmol/l)	Alkalinity	Total number of *B. marginata* shells produced (all sizes)	Size fraction (‰ PDB)	$\delta^{18}O_f$ (‰ PDB)	$\delta^{18}O_f$-$\delta^{18}O_w$
Petri dish system PD							
7.9 ± 0.1	35.9 ± 0.1	7.92 ± 0.06	2449 ± 36	402	< 150	2.02	1.69
					< 150	1.95	1.62
					150–200	2.09	1.76
					150-200	2.12	1.79
10.2 ± 0.1	35.8 ± 0.1	7.92 ± 0.06	2451 ± 43	593	< 150	1.47	1.14
					< 150	1.50	1.17
					150–200	1.60	1.27
					150–200	1.69	1.36
					200–250	1.53	1.20
12.7 ± 0.2	35.9 ± 0.1	7.93 ± 0.06	2450 ± 52	585	< 150	0.97	0.64
					< 150	0.95	0.62
					150–200	1.00	0.67
					150–200	1.05	0.72
14.7 ± 0.1	35.9 ± 0.1	7.94 ± 0.07	2454 ± 46	445	< 150	0.46	0.13
					< 150	0.42	0.09
					150–200	0.60	0.27
13.0 ± 0.1	35.9 ± 0.1	7.91 ± 0.05	2412 ± 15	890	< 150	0.89	0.56
					< 150	1.08	0.75
					< 150	0.77	0.44
					150–200	0.93	0.60
					150–200	1.00	0.67
					150–200	1.07	0.74
					200–250	1.01	0.68
					200–250	1.07	0.74
Closed system CSI							
7.9 ± 0.1	35.8 ± 0.1	7.94 ± 0.04	2454 ± 24	304	< 150	2.08	1.75
					> 150	1.91	1.58
					> 150	2.17	1.84
10.1 ± 0.1	35.8 ± 0.1	7.96 ± 0.04	2457 ± 21	777	< 150	1.31	0.98
					< 150	1.45	1.12
					=150	1.39	1.06
					=150	1.46	1.13
					=150	1.52	1.19
					150–200	1.56	1.23
					150–200	1.44	1.11
					150–250	1.44	1.11
					150–250	1.54	1.21
					200–250	1.60	1.27
					200–250	1.57	1.24
					200–250	1.63	1.30
					> 250	1.61	1.28
					> 250	1.66	1.33
					> 250	1.56	1.23
12.7 ± 0.1	35.9 ± 0.1	7.98 ± 0.04	2473 ± 34	719	< 150	0.79	0.46
					=150	0.82	0.49
					=150	0.84	0.51
					> 150	0.98	0.65
					150–200	1.01	0.68
					200–250	1.12	0.79
					200–250	1.13	0.80
					> 250	1.17	0.84
14.7 ± 0.1	35.8 ± 0.1	7.96 ± 0.04	2473 ± 31	569	< 150	0.49	0.16
					150–200	0.61	0.28
					150–200	0.62	0.29
					150–200	0.63	0.30
					200–250	0.60	0.27
					200–250	0.79	0.46
					200–250	0.47	0.14
					> 250	0.73	0.40
					> 250	0.75	0.42

Table 1. Continued.

Temperature (°C)	Salinity	pH (µmol/l)	Alkalinity	Total number of *B. marginata* shells produced (all sizes)	Size fraction (‰ PDB)	$\delta^{18}O_f$ (‰ PDB)	$\delta^{18}O_f$-$\delta^{18}O_w$
Closed system CSII							
4.1 ± 1.1	35.8 ± 0.1	7.80 ± 0.07	2528 ± 13	110	< 100	2.77	2.44
6.0 ± 0.5	35.8 ± 0.1	7.80 ± 0.08	2524 ± 12	261	< 100	2.31	1.98
					< 100	2.07	1.74
					100–150	2.21	1.88
9.3 ± 0.7	35.8 ± 0.1	7.78 ± 0.09	2524 ± 13	2461	< 100	1.85	1.52
					< 100	1.66	1.33
					100–150	1.78	1.45
					100–150	1.65	1.32
					100–150	1.57	1.24
					=150	1.71	1.38
					=150	1.72	1.39
					150–200	1.78	1.45
					150–200	1.73	1.40
11.6 ± 0.3	35.8 ± 0.1	7.80 ± 0.10	2525 ± 11	567	100–150	1.21	0.88
					100–150	1.06	0.73
					100–150	1.06	0.73
					=150	1.13	0.80
					=150	1.05	0.72
					150–200	1.06	0.73
					150–200	1.07	0.74
					200–250	1.27	0.94
					200–250	1.08	0.75
17.2 ± 0.2	35.8 ± 0.1	7.77 ± 0.10	2521 ± 19	17	150–200	−0.07	−0.40
19.3 ± 0.1	35.8 ± 0.1	7.80 ± 0.09	2519 ± 16	84	150-200	−0.44	−0.77

of our experiments (4–19 °C), we obtain a maximum temperature offset of 0.2 °C compared to the quadratic equation. This variation corresponds to a $\delta^{18}O_f$ bias of 0.05‰ which is equivalent to the precision of the mass-spectrometer. The coefficient of determination (R^2) and the standard errors on the slope and intercept are indicated for each equation.

3 Results and discussion

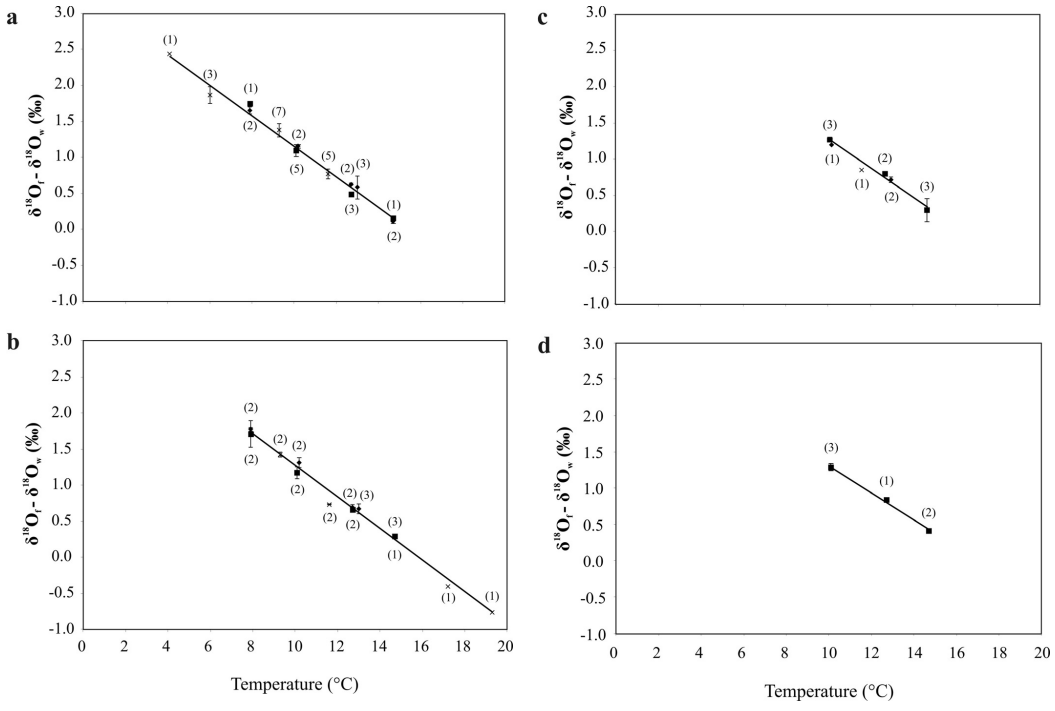
3.1 Influence of temperature on the δ^{18}O of cultured foraminifera

Knowing that shell size may have an effect on isotope ratio in foraminifera (Spero and Lea, 1996; Bemis et al., 1998; Elderfield et al., 2002; Schmiedl et al., 2004), our data were treated according to four different size fractions to consider this possible effect on *B. marginata*: ≤150 µm, 150–200 µm, 200–250 µm and >250 µm. For each of the four size fractions, we plotted the oxygen isotopic composition of the shell of *B. marginata* ($\delta^{18}O_f - \delta^{18}O_w$) as a function of the different temperatures tested in the experiments (Fig. 1a–d, Table 1). The $^{18}O/^{16}O$ composition of *B. marginata* appears similar for the 3 experimental protocols (CS_I, CS_{II} and PD) for a given temperature and given size fraction (Fig. 1). For the ≤150 and 150–200 µm size fractions, where sufficient data are available, we used Lin's test (Lin, 1989) to estimate the concordance of the regression lines for the three systems. For all cases, we obtained concordance correlation coefficients above 0.990, confirming the high degree of similarity of the data obtained with the three systems. Therefore, we conclude that the pH decrease in CS_{II} did not cause a significant shift of the $\delta^{18}O$ of foraminifera calcified in these experiments. Since the $\delta^{18}O$ of *B. marginata* appears to be independent of the applied protocol, in the following text we will no longer distinguish the three experimental set-ups.

The linear equations which best describe the relationship between temperature and $\delta^{18}O$ of foraminiferal tests entirely calcified under controlled laboratory conditions are, for the four different size fractions (Fig. 1):

$$T(^{\circ}C) = 15.25\ (\pm 0.17) - 4.54\ (\pm 0.14) \cdot (\delta^{18}O_f - \delta^{18}O_w) \quad (1)$$

for $\leq 150\,\mu m$



e	Size fraction	Temperature range	T (°C) = b + a * ($\delta^{18}O_f$-$\delta^{18}O_w$)		R^2
			a	b	
Equation (1)	≤150 µm	4.1-14.7°C	– 4.54 (± 0.14)	15.25 (± 0.17)	0.97
Equation (2)	150-200 µm	7.9-19.3°C	– 4.49 (± 0.13)	15.73 (± 0.14)	0.98
Equation (3)	200-250 µm	10.1-14.7°C	– 4.61 (± 0.37)	16.00 (± 0.33)	0.93
Equation (4)	>250 µm	10.1-14.7°C	– 5.31 (± 0.23)	16.93 (± 0.23)	0.99

Fig. 1. Experimental calibration equations of temperature versus $\delta^{18}O_f - \delta^{18}O_w$ of cultured specimens of *B. marginata*. Averages and standard deviations are presented separately for the ≤150 µm **(a)**, 150–200 µm **(b)**, 200–250 µm **(c)**, and > 250 µm **(d)** shell size fractions. The number of measurements for each average value is indicated in parenthesis. Different symbols correspond to the three systems: PD (diamonds), CS_I (squares) and CS_{II} (crosses). The calibration equations summarised in **(e)** are based on all data from each size fractions (for all equations $p < 0.001$).

$$T(^{\circ}\mathrm{C}) = 15.73\ (\pm 0.14) - 4.49\ (\pm 0.13) \cdot (\delta^{18}O_f - \delta^{18}O_w) \quad (2)$$
$$\text{for } 150 - 200\,\mu\mathrm{m}$$

$$T(^{\circ}\mathrm{C}) = 16.00\ (\pm 0.33) - 4.61\ (\pm 0.37) \cdot (\delta^{18}O_f - \delta^{18}O_w) \quad (3)$$
$$\text{for } 200 - 250\,\mu\mathrm{m}$$

$$T(^{\circ}\mathrm{C}) = 16.93\ (\pm 0.23) - 5.31\ (\pm 0.23) \cdot (\delta^{18}O_f - \delta^{18}O_w) \quad (4)$$
$$\text{for } > 250\,\mu\mathrm{m}$$

Equations (1, 2 and 3) exhibit similar slopes considering the standard errors on the slope estimates. For these three size fractions, the relative influence of temperature on the oxygen isotopic composition of *B. marginata* is -0.22‰ °C^{-1}. For the > 250 µm size fraction, the linear regression between $\delta^{18}O_f - \delta^{18}O_w$ and temperature presents a steeper slope (Eq. 4). However, the linear regression for this size fraction is less well defined than that obtained for the smaller size fractions, since data are available only for three different temperatures and only few individuals attained a size larger than 250 µm. Further experimental work is needed to refine this (Eq. 4), which we will not consider in the remaining part of this paper.

3.2 Influence of shell size on the δ^{18}O of cultured foraminifera

Interestingly, there is an increase in the intercept values with increasing size fraction (15.25, 15.73 and 16.00 respectively for the size fractions ≤150, 150–200 and 200–250 µm; Fig. 1 e), indicating a shift towards higher δ^{18}O values with increasing size. In Fig. 2, individual δ^{18}O measurements are

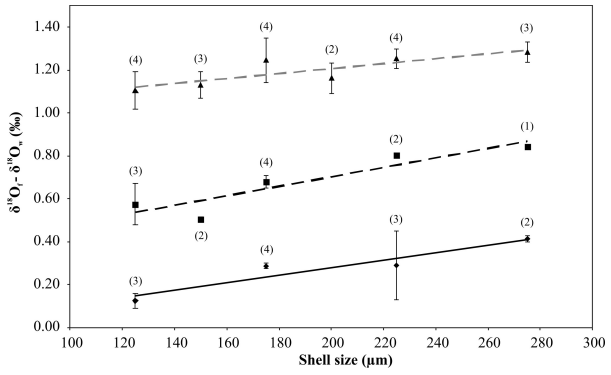


Fig. 2. Shell size effect on the oxygen isotopic composition ($\delta^{18}O_f - \delta^{18}O_w$) of *B. marginata* calcified in culture at 10.2 (triangles), 12.7 (squares) and 14.7 °C (diamonds). The number of measurements for each average value is indicated in parenthesis. The linear regressions are: y = 0.0012 x + 0.9745 (R^2 = 0.40; $p = 0.003$; gray dashed line) at 10.2 °C; y = 0.0022 x + 0.2655 (R^2 = 0.71; $p = 0.001$; black dashed line) at 12.7 °C; and y = 0.0017 x + 0.0588 (R^2 = 0.57; $p = 0.005$; black line) at 14.7 °C.

presented as a function of test size for the three temperatures for which we had a sufficient amount of different size fractions to obtain a reliable regression equation ($p < 0.01$). Figure 2 shows that at 10.2, 12.7 and 14.7 °C, the δ^{18}O of the foraminiferal tests increases by 0.0012–0.0022‰ µm^{-1}, with determination coefficients (R^2) between 0.4 and 0.7 ($p < 0.01$). The calcification rate plays an important role in the fractionation of the organisms since higher growth rates will result in a more depleted δ^{18}O and δ^{13}C (McConnaughey, 1989a, 1989b). This is due to a "kinetic effect" which corresponds to the discrimination against heavy C and O isotopes during hydration ($CO_2 + H_2O \rightarrow H_2CO_3$) and hydroxylation ($CO_2 + OH^- \rightarrow HCO_3^-$) of CO_2. Because younger foraminifera calcify faster (Berger et al., 1978), they may not attain equilibrium in the calcification reservoir before crystallisation, which would result in the production of more negative δ^{18}O and δ^{13}C values. Such a possibility was earlier proposed by Turner (1982). For benthic foraminifera, ecological experiments tend to prove that growth of specimens is not uniform and chambers addition is faster during the first ontogenetic stages (Bradshaw, 1957, 1961; Hemleben and Kitazato, 1995; Stouff et al., 1999). This has also been observed for *Bulimina marginata* (Barras et al., 2009).

The influence of size on oxygen isotopic composition is well established for planktonic foraminifera (Spero and Lea, 1996; Bemis et al., 1998; Elderfield et al., 2002), whereas previous field-based studies of size-dependent trends in benthic foraminiferal isotopic values have been inconclusive (Vincent et al., 1981; Dunbar and Wefer, 1984; Grossman, 1987; Corliss et al., 2002). Generally, in these studies, benthic foraminifera do not show a significant change in δ^{18}O with size. However, some authors observed an ontogenetic effect on the oxygen isotopic fractionation of *Bulimina aculeata/marginata* shells obtained in laboratory experiments (McCorkle et al., 2008; Filipsson et al., 2010) and living and dead *Uvigerina mediterranea* from the western Mediterranean Sea (Schmiedl et al., 2004). Schmiedl et al. (2004) found a 0.3–0.4‰ δ^{18}O enrichment over a size range of 175 to 1250 µm. This enrichment was particularly important in the early growth stages (100–300 µm) and became weaker for adult forms, which might be explained by the decreasing metabolic rates towards more adult life stages. If we compare the slope of their logarithmic correlation equation for these younger stages (the size fraction we studied) with our data, their δ^{18}O versus test size curve has an average slope of about 0.001‰ µm^{-1} which is similar to the size effect found in our experiments. Even if adult specimens of *B. marginata* are smaller than adult specimens of *U. mediterranea*, it is probable that the specimens measured in our experiments were not large enough to reach the stable isotopic composition typical of larger specimens, as observed for *U. mediterranea* (Schmiedl et al., 2004). Either our specimens were still growing when the experiments were stopped, or they died before attaining the "adult" stage. It would be useful in future experiments to grow living *B. marginata* during longer time than in our experiments and try to obtain larger size fractions.

On the basis of all our 83 δ^{18}O measurements performed on specimens of *B. marginata* which totally calcified under controlled conditions (Table 1), we applied a multiple regression that takes into account δ^{18}O of the shells, calcification temperature (4–19 °C) as well as test size (50–300 µm). According to this multiple regression, the averaged size effect on δ^{18}O composition of *B. marginata* is 0.0014‰ µm^{-1}. It appears therefore that an ontogenetic effect on oxygen isotope fractionation exists also for benthic foraminifera and cannot be neglected in paleoceanographic studies. Since the regression lines of $\delta^{18}O_f - \delta^{18}O_w$ versus test size are more or less parallel for the tested temperatures, we conclude that the mechanism responsible for this small ontogenetic effect is independent of calcification temperature. We recommend performing measurements in a size range not larger than 50 µm to fully exploit the 0.07‰ accuracy of mass-spectrometric analyses.

3.3 Comparison with equilibrium calcite as defined by Kim and O'Neil (1997)

Among the numerous paleotemperature equations published since the 1950's, Kim and O'Neil (1997) reinvestigated the relationship of O'Neil (1969) based on inorganically precipitated calcite for a temperature range between 10 and 40 °C (Eq. 5).

$$T(°C) = 16.1 - 4.64 \cdot (\delta^{18}O_f - \delta^{18}O_w) + 0.09 \cdot (\delta^{18}O_f - \delta^{18}O_w)^2 \quad (5)$$

We compared our experimental calibration equations with the Kim and O'Neil (1997) equation because this equation was established under controlled laboratory conditions as in

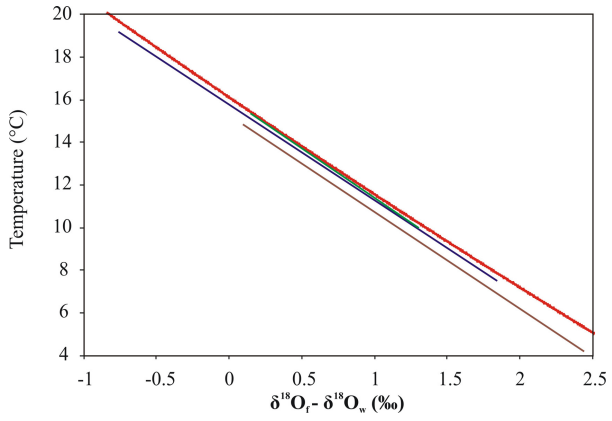


Fig. 3. Comparison of our experimental calibration equation with the theoretical equation for equilibrium calcite of Kim and O'Neil (1997). The brown, blue and green lines represent the calibration equations of cultured *B. marginata* from ≤150, 150–200 and 200–250 µm size fractions, respectively. The quadratic equation derived from Kim and O'Neil (1997) relationship is represented by the red line.

our study, and measurements were performed on inorganic calcite, free of vital effects. The three experimental regression curves we determined for size fractions smaller than 250 µm exhibit similar slopes as the least square regression line applied to the quadratic relationship of Kim and O'Neil (1997) over the studied temperature range (Fig. 3). Therefore, the influence of temperature on the δ^{18}O of calcite is similar, and independent of test size. Furthermore, the offsets of the foraminiferal curves with respect to the inorganic carbonate curve are very small. Regression lines (2) and (3) fit well with the Kim and O'Neil (1997) equation (taking into account the standard errors), suggesting that for the 150–200 and 200–250 µm size classes, the biological effect is negligible. Over the investigated temperature range of 4 to 19 °C, the difference on the temperature estimates between Eqs. 2, 3 and the Kim and O'Neil equation is at most 0.7 °C. However, the calibration equation of cultured *B. marginata* for the 200–250 µm fraction is closer to Kim and O'Neil relationship than the equations derived for smaller size fractions. Additional measurements are necessary to accurately study the δ^{18}O of size fractions larger than 250 µm.

4 Conclusions

The new protocols developed for this study allowed us to obtain reproduction and calcification of the deep-sea benthic foraminifer *Bulimina marginata* under controlled conditions at 12 different temperatures between 4 and 19 °C. In general, a 1 °C decrease in calcification temperature increases the δ^{18}O of *Bulimina marginata* by +0.22‰, irrespective of the size fraction and culture setup considered. This effect is similar to the thermodynamical effect observed for inorganic calcite. However, our data show a small but conspicuous ontogenetic effect on δ^{18}O values of about 0.0014‰ µm^{-1} that should be taken into account in order to produce accurate paleoclimatic reconstructions. *Bulimina marginata* specimens with a test length between 150 and 250 µm calcify very close to the equilibrium calcite as defined by Kim and O'Neil (1997). Finally, these experiments, leading to reliable data, proved that the foraminiferal treatment protocols developed for this study could be applied in future studies to investigate the impact of other physico-chemical parameters (salinity, carbonate chemistry…) on benthic foraminiferal shell composition (isotopes, trace metals…).

Acknowledgements. These studies were supported by CEA, CNRS, UVSQ and the Region Pays de la Loire. We are grateful to M. Gaultier for her precious technical support in the laboratory and to G. Hunault for his help with statistics. We thank B. Minster, F. Vimeux and A. Mackensen for seawater δ^{18}O measurements and careful discussions on calibration of laboratory standards, and F. Dewilde and F. Manssouri for making foraminiferal δ^{18}O measurements with their usual accuracy. We would like to thank L. J. de Nooijer and G. Schmiedl for their constructive reviews and useful comments. This is LSCE contribution number 4208.

Edited by: J. Bijma

The publication of this article is financed by CNRS-INSU.

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