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Geochimica et Cosmochimica Acta 75 (2011) 520–532
Geochimica et Cosmochimica Acta
www.elsevier.com/locate/gca

Interindividual variability and ontogenetic effects on Mg and Sr incorporation in the planktonic foraminifer *Globigerinoides sacculifer*

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Received 30 November 2009; accepted in revised form 5 October 2010; available online 16 October 2010

Abstract

In order to investigate the interindividual and ontogenetic effects on Mg and Sr incorporation, magnesium/calcium (Mg/Ca) and strontium/calcium (Sr/Ca) ratios of cultured planktonic foraminifera have been determined. Specimens of *Globigerinoides sacculifer* were grown under controlled physical and chemical seawater conditions in the laboratory. By using this approach, we minimised the effect of potential environmental variability on Mg/Ca and Sr/Ca ratios. Whereas temperature is the overriding control of Mg/Ca ratios, the interindividual variability observed in the Mg/Ca values contributes 2–3 °C to the apparent temperature variance. Interindividual variability in Sr/Ca ratios is much smaller than that observed in Mg/Ca values. The variability due to ontogeny corresponds to −0.43 mmol/mol of Mg/Ca ratio per chamber added. This translates into an apparent decrease of ~1 °C in Mg/Ca-based temperature per ontogenetic (chamber) stage. No significant ontogenetic effect is observed on Sr incorporation. We conclude that the presence of a significant ontogenetic effect on Mg incorporation can potentially offset Mg/Ca-based temperature reconstructions. We propose two new empirical Mg/Ca-temperature equation based on Mg/Ca measurements of the last four ontogenetic (chamber) stages and whole foraminiferal test: $Mg/Ca = (0.55(\pm 0.03) - 0.0002(\pm 4 \times 10^{-5})\ MSD)\, e^{0.089T}$ and, $Mg/Ca = (0.55(\pm 0.03) - 0.0001(\pm 2 \times 10^{-5})\ MSD)\, e^{0.089T}$, respectively, where MSD corresponds to the maximum shell diameter of the individual.

1. INTRODUCTION

Planktonic foraminifera are commonly used for the reconstruction of sea surface temperature (SST) (Nürnberg et al., 2000; Elderfield and Ganssen, 2000; Anand et al., 2003; McKenna and Prell, 2004; McConnell and Thunell, 2005). Specifically, trace element (e.g. Mg) and stable isotope compositions of their carbonate tests are used to reconstruct SST (Nürnberg et al., 1996a; Lea, 1999; Nürnberg et al., 2000; Erez, 2003; McKenna and Prell, 2004; Groeneveld et al., 2008), which indicates that the composition of foraminiferal carbonate tests can be used as a proxy for various physical and chemical parameters of seawater. Over the past two decades, several studies have shown that the incorporation of trace elements into the carbonate test is not only affected by changes in the parameter to which they systematically and reliably respond, but is also influenced by other parameters. Laboratory culture studies conducted in the last decade show that salinity, pH and carbonate concentration $[CO_3^{2-}]$, among other parameters, affect the composition of planktonic foraminiferal tests

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doi:10.1016/j.gca.2010.10.006

(Spero et al., 1997; Lea et al., 1999; Russell et al., 2004; Mortyn et al., 2005; Kısakürek et al., 2008; Dueñas-Bohórquez et al., 2009). In addition, so-called "vital effects", which refer to biologically controlled processes, are responsible for the additional variability found in foraminiferal trace metal incorporation (Nürnberg et al., 1996a; Erez, 2003; Eggins et al., 2003; Sadekov et al., 2005; Bentov and Erez, 2006; Kunioka et al., 2006).

The aim of the present study is to determine magnitude and mechanisms behind interindividual and ontogenetic effects on planktonic foraminiferal Mg and Sr incorporation under controlled physical and chemical seawater conditions. Quantifying the magnitude of these effects is of great importance since, for instance, the use of interindividual variability to reconstruct seasonality is starting to increase (Sadekov et al., 2008). We have specifically chosen *Globigerinoides sacculifer* (Brady) as it is a key, mixed layer dwelling, species that is widely used for sea surface temperature reconstructions.

2. METHODOLOGY

2.1. Collection and culturing of foraminifera

Specimens of the planktonic, symbiont-bearing foraminifera *Globigerinoides sacculifer* (Fig. 1a–c) were collected from surface waters (2–6 m depth) by scuba diving, 10 km off the southwest coast of Puerto Rico (17° 54′ 46″N, 66° 58′ 44″W), between April and June 2006. Specimens were brought back to the marine station at Magueyes Island (Department of Marine Sciences, University of Puerto Rico at Mayaguez), La Parguera, Puerto Rico where the culture experiments were setup. Surface seawater for culturing the foraminifera was collected at the dive site and filtered over 0.25 μm polycarbonate membrane filters.

We identified and measured individual specimens using an inverted light microscope and transferred them to 120-ml Pyrex bottles filled with seawater at *in situ* temperature and salinity conditions (26 °C and 36, respectively). These bottles were incubated in thermostated water baths (26 ± 0.5 °C) and kept at a constant light intensity of 353 μE/m^2/s (12-h light/12-h dark cycle). Specimens were fed every third day with a 1-day-old *Artemina salina* (nauplius), starting on the day of collection. For these culture experiments, we followed the procedures outlined in Bijma et al. (1990) in order to minimize any stressful conditions that could potentially affect foraminiferal element incorporation.

Specimens were monitored daily and usually underwent gametogenesis between 7 and 14 days after collection. Then they were removed from the solutions, rinsed with deionised water and archived for later analysis. Foraminifera that built new chambers in the laboratory were identified by comparing their real size (different from sieving size which corresponds to F-1 chamber size; Bijma and Hemleben, 1994) at collection with the real size of the test after gametogenesis (spines discarded). Foraminifera used for element analyses formed 1–3 additional chambers during the incubations. In order to increase the number of data points for this study, Mg/Ca ratios from specimens grown under different temperature and salinity conditions (Table EA4) were normalized to values at 26 °C and salinity of 36. This was achieved by using existing Mg/Ca-temperature and Mg/Ca-salinity calibrations (Nürnberg et al., 1996b; Dueñas-Bohórquez et al., 2009, respectively) (Table EA5). Sr/Ca ratios from specimens grown under different temperatures and salinities were not normalized since no significant effect of these two parameters was found on Sr/Ca ratios (see Section 3). This applies to the temperature and salinity intervals considered here (23–29 °C, according to Sr/Ca-temperature calibration of Kısakürek et al. (2008); and 30–39 salinity units, according to Sr/Ca-salinity calibration of Dueñas-Bohórquez et al. (2009) (Table EA5).

Results presented here are based on analysis of three morphotypes of *G. sacculifer*: (1) Normal morphotype (NOR, last chamber is larger than and similar in shape to the previous one); (2) Sac-like morphotype (SAC, last chamber with a very distinctive cone-like shape) and, (3) Kummerform morphotype (KUM, last chamber is equal to or smaller than the previous chamber) (Hemleben et al., 1987) (Fig. 1a–c).

According to the previously established correlation between chamber number and maximum shell diameter (MSD or shell size) of *G. sacculifer* (Hemleben and Bijma, 1994), the final (F) chamber corresponds to chamber-stage 19 (in a few cases it corresponds to chamber-stage 20). The penultimate chamber (F-1) corresponds to chamber-stage 18 and, F-2 chambers correspond to chamber-stage 17.

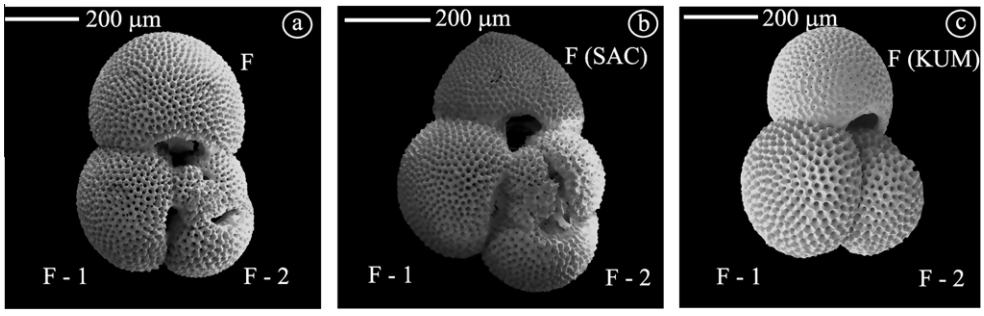

Fig. 1. *Globigerinoides sacculifer* analysed by Laser ablation-ICP–MS and its three different morphotypes based on the size and shape of the last chamber. (a) Normal (NOR), (b) Sac-like (SAC) and, (c) Kummerform (KUM). F = final chamber or chamber-stage 19 (in few cases chamber-stage 20); F-1 = penultimate chamber or chamber-stage 18; F-2 = third chamber/ chamber-stage 17 (older than F and F-1).

2.2. Carbonate chemistry of the culture solutions

Carbonate parameters of the seawater used for the culture experiments were calculated using the CO2SYS software (version 01.05; Lewis and Wallace, 1998). Salinity, total alkalinity and pH (NBS scale) of the solutions were measured at the beginning and at the end of the experiments which usually lasted seven to ten days (Table 1).

Seawater samples were collected and brought to the laboratory at Utrecht University to analyse Dissolved Inorganic Carbon (DIC) using a Total Organic Carbon Analyser (Shimadzu, Model TOC-5050A). DIC results from this analysis and DIC values calculated via the CO2SYS software were not significantly different (results are presented in electronic annex EA, Table EA1).

2.3. Sample preparation and analysis

Specimens that grew new chambers were cleaned in a sodium hypochlorite bath (NaClO 5%) for 20 min and rinsed 3 times with deionised water, carefully pipetting off the supernatant. Mg/Ca ratios were measured by laser-ablation inductively coupled plasma-mass spectrometry (LA–ICP–MS, Micromass Platform). This technique allows us to measure trace element concentrations of individual chambers from single specimens several times (Reichart et al., 2003).

The chambers were ablated using a 193 nm laser (GeoLas 200Q Excimer) in a helium flushed ablation chamber which was coupled to the ICP-MS. Pulse repetition rate was set at 6 Hz with an energy density at the sample surface of 1 J/cm^2. Ablation craters were 80 μm in diameter and the ablated calcite was analysed with respect to time. The wall of the carbonate tests of most of the chambers was ablated over the full thickness (Fig. 2). Thus the measurements of Mg/Ca and Sr/Ca ratios given in the present study correspond to the mean values of the different layers constituting the test wall (Sadekov et al., 2005). Calibration was performed against US National Institute of Standards and Technology (NIST) SRM 610 glass with Ca as an internal standard. Using calcium as an internal standard is ideal as this element is present at a constant concentration of 40% in calcium carbonate ($CaCO_3$). This also allows direct comparison with the more traditional wet chemical analyses (Reichart et al., 2003). A collision and reaction cell was used to give improved results by reducing spectral interferences on the minor isotopes of Ca (^{42}Ca, ^{43}Ca, and ^{44}Ca). The NIST SRM 610 glass reference material was measured with a higher energy density (4 J/cm^2) than the calcite samples. To check whether using different ablation energy biases the analyses, a matrix matched standard was included. This standard is a homogeneous calcite crystal (Icelandspar) that was analysed by LA–ICP–MS using four different ablation energy densities (results in EA, Table EA2).

Results show that Mg and Sr values do not significantly vary when different energy densities were used to ablate the calcite crystal. Subsamples taken from this calcite crystal were also dissolved in ultra clean HNO_3 (Merck) and subsequently analysed using an ICP–AES (Spectro CIROS CCD). A comparison between these two analyses shows that, although a different energy density was used for the glass and calcite standard, Mg and Sr concentrations are statistically identical (results in EA, Table EA3). Based on repetitive analyses of the calcite standard throughout the analytical period during which planktonic specimens were measured, relative precission of the LA–ICP–MS analyses for Mg and Sr were around 3% (748 ± 23 ppm) and 3.6% (193 ± 7 ppm), respectively. Monitoring simultaneously ^{42}Ca, ^{43}Ca and ^{44}Ca showed isotopic ratios expected on basis of their natural relative abundances.

Accuracy for each individual analysis was calculated using the Glitter software, which was also used to calculate elemental concentrations (Glitter, LA–ICP–MS Data Reduction and Display, GEMOC, CSIRO, Maquarie Research Limited, 1999–2000). The intervals of the acquired data used to calculate concentrations were selected avoiding sections with high Al and/or Pb counts. Although the foraminifera were never in contact with sediments as a source for contamination, this ensures that an (unknown) phase does not introduce errors in the trace metal analyses.

Comparisons of Mg/Ca and Sr/Ca ratios between chamber-stages (ontogenetic effect) of *G. sacculifer* were done by Analysis of Variance (ANOVA) once it was determined the data met the basic assumptions of a normal distribution. A *post hoc* test (Hochberg) (Field, 2009) was used to compare all different combinations of data groups and find significant differences between them. *t*-tests were performed to determine significant differences between the Mg/Ca-based temperature means.

3. RESULTS

3.1. Interindividual variability

The Mg/Ca and Sr/Ca ratios from specimens that grew under controlled conditions in the laboratory (constant temperature of 26 °C and a salinity of 36) are shown in Fig. 3. Each chamber stage analysed is characterized by a large range of Mg/Ca values; for instance, from 3 to 8.4 mmol/mol in the case of chamber 17 (Fig. 3a). Variability in Mg/Ca ratios measured per chamber stage is relatively large and ranges from 0.63 to 1.28 mmol/mol (±1 standard deviation; Table 2). Ratios of Sr/Ca show a narrower range of values per chamber-stage than Mg/Ca ratios

Table 1
Experimental conditions.[a]

Salinity	Alkalinity (μmol/kg SW)	pH (NBS scale)	Mean $[CO_3^{2-}]$ (μmol/kg SW)	DIC (μmol/kg SW)	Ωc
36 ± 0.3	2391 ± 35.4	8.22 ± 0.02	246 ± 12	2156 ± 146	5.9 ± 0.4

[a] Culture experiments were carried out at $T = 26$ °C and a light intensity = 353 μEm^{-2} s^{-1}. Mean $[CO_3^{2-}]$ and DIC were calculated from alkalinity and pH measurements using the program CO2sys (Lewis and Wallace, 1998, version 01.05), with the CO_2 constants of Mehrbach et al. (1973) refit by Dickson and Millero (1987), and K_{SO4} from Dickson (1990). Ωc refers to saturation state for calcite.

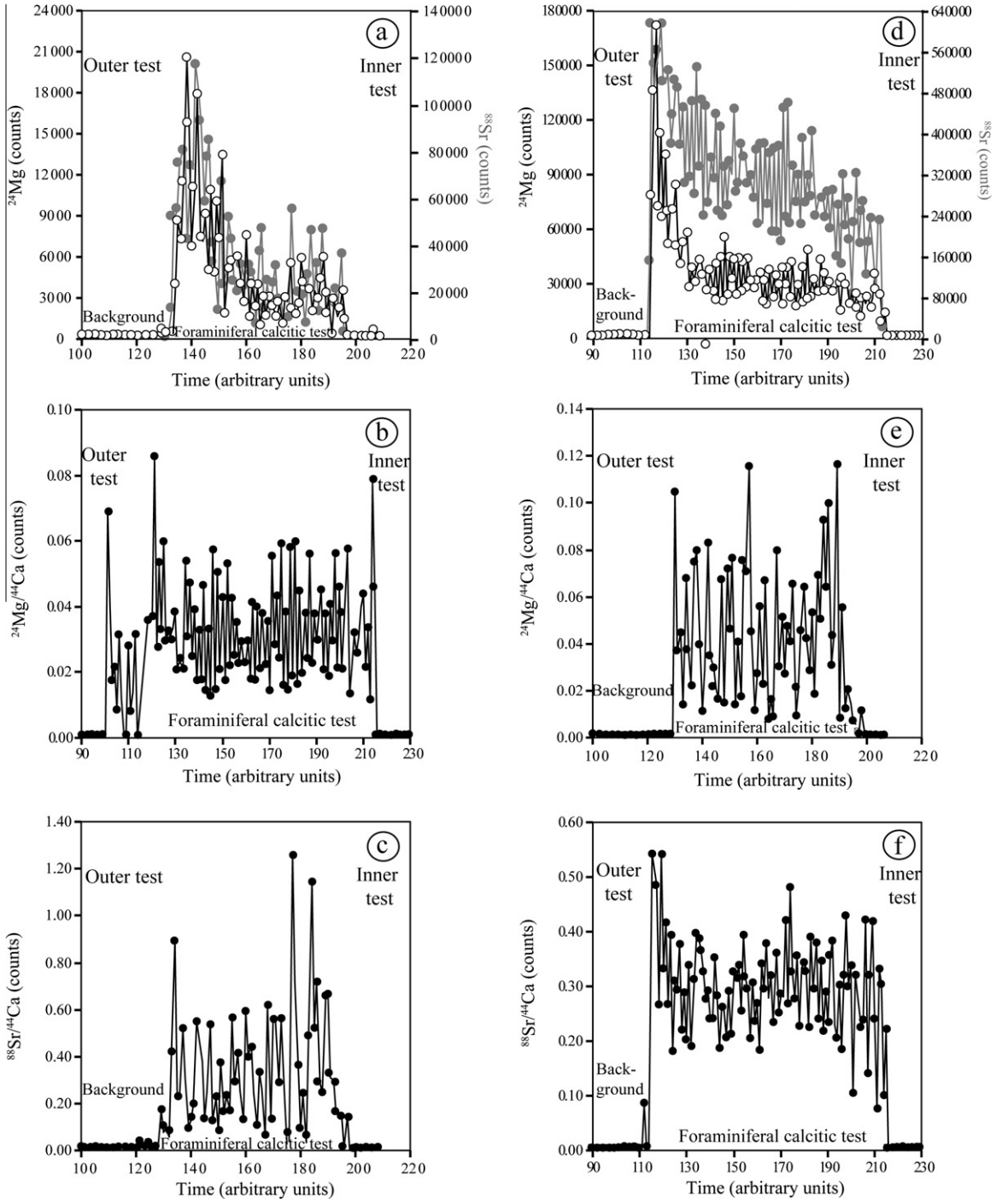

Fig. 2. Laser ablation profiles of *G. sacculifer:* ^{24}Mg, ^{88}Sr, Mg/Ca and Sr/Ca (counts) of final chamber (F or chamber stage 20) (a–c); ^{24}Mg, ^{88}Sr, Mg/Ca and Sr/Ca (counts) of penultimate chamber (F-1 or chamber stage 19) (d–f). Open circles correspond to ^{24}Mg; grey circles correspond to ^{88}Sr; closed circles correspond to ^{24}Mg/Ca and ^{88}Sr/Ca.

(Fig. 3b) and also a much smaller variability in the Sr/Ca ratio means between chambers. However, some interindividual variability is still observed (Table 2 and Fig. 3b).

3.2. Ontogenetic variations of Mg/Ca and Sr/Ca

Results of this section are based on the combined data from all three morphotypes of *G. sacculifer* identified in the present study (SAC, NOR and KUM) (Fig. 1). Chamber stage18 has the highest average Mg/Ca value, followed by chambers 17, 19 and 20. Chamber 17, which is the oldest chamber grown in culture, has a much wider range of Mg/Ca ratios, encompassing most of the values found in the rest of the chambers analysed (Fig. 3a). In addition, the Mg/Ca values of *G. sacculifer* slowly decrease from the oldest (chamber 17) to the last chamber (newest) grown

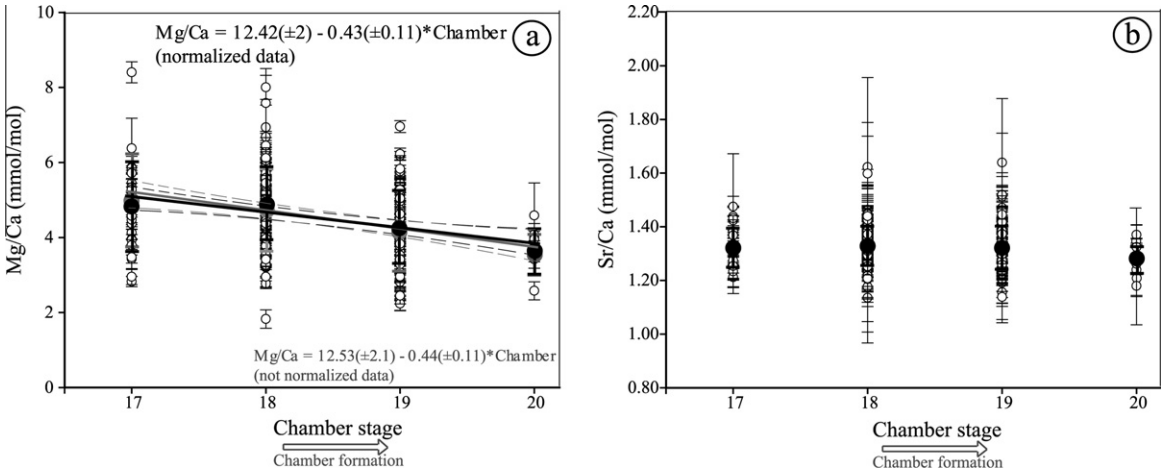

Fig. 3. Foraminiferal Mg/Ca (a) and Sr/Ca (b) ratios vs. ontogenetic stage (chamber stage). Open circles correspond to normalized Mg/Ca ratios to 26 °C and 36 salinity units; normalized Mg/Ca means and standard deviations are shown in black; not normalized Mg/Ca means and standard deviations for every ontogenetic stage are shown in grey. Every Mg/Ca and Sr/Ca point corresponds to the average value of LA-analyses performed on a single specimen. Error bars indicate ±1 standard deviation. The 95% confidence limits of the curve fit are shown by dashed lines.

Table 2
Summary of mean Mg/Ca and Sr/Ca ratios for four ontogenetic stages of *G. sacculifer*.

Chamber stage	n[a]	Mg/Ca (mmol/mol)	SD[b] Mg/Ca	n[a]	Variability of Mg/Ca (%)	Sr/Ca (mmol/mol)	SD[b] Sr/Ca
17	23	4.75	1.28	23	27	1.34	0.07
18	94	4.82	1.09	99	23	1.35	0.08
19	77	4.13	1.07	77	26	1.34	0.09
20	7	3.76	0.63	9	17	1.30	0.06

Values are given with ±1 standard deviations.
[a] n refers to the number of specimens used to calculate the mean and the standard deviation.
[b] Standard deviation.

in culture (chamber 20) (Table 2 and Fig. 3a) despite the relatively large interindividual variability (Fig. 3). In contrast, the Sr/Ca ratios are very similar for the four chambers analysed (Table 2 and Fig. 3b).

4. DISCUSSION

Results presented here are based on combined data from the three morphotypes of *G. sacculifer* identified during this study (NOR, SAC and KUM). We use this approach since the Mg and Sr incorporation into the test walls of these three morphotypes is not significantly different (Anand et al., 2003). Normalized and not normalized Mg/Ca ratios are plotted together (Fig. 3a) and show that there are no significant differences between the adjusted (normalized) and the original ratios. Therefore, the normalized data will be used throughout the rest of the paper.

4.1. Interindividual variability in Mg/Ca ratios

4.1.1. Possible causes of interindividual variability

Variability in Mg incorporation between individuals grown under controlled physical and chemical conditions (Table 1) is too large to be explained by analytical uncertainties alone (Fig. 3). The standard deviations (SDs) shown in Table EA5 represent the Mg/Ca variability found between measurements performed in different sections of one chamber. This variability may represent the differential Mg banding within one chamber wall.

When we compare these SDs to the SDs from the Mg/Ca interindividual variability (Table 2), we observe that the latter are much larger. This means that the contribution of Mg/Ca intra-chamber differences to the final Mg/Ca variability is minor when compared to the contribution of the interindividual changes.

The planktonic specimens analysed in this study were grown under constant and controlled laboratory conditions which means that neither physical and/or chemical parameters of the seawater are responsible for the variation in Mg/Ca ratios between individuals. Post depositional dissolution of the carbonate test is also discarded as a source of variability between specimens.

High interindividual variability has also been observed in core-top and plankton samples of *G. ruber* (Sadekov et al., 2008) in a magnitude similar to the one we report here (0.6–1.1 mmol/mol; present study 0.6–1.3 mmol/mol; Table 2). Several authors (Eggins et al., 2003; Sadekov et al., 2008; Yu and Elderfield, 2008) have suggested a differential development of Mg/Ca banding between individuals as the cause of this Mg variation. Differences in the thickness of low and high-Mg layers between individuals might also be the source of this interindividual variation.

The high interindividual variability observed in *G. sacculifer* might alternatively be caused by differences in the density of symbionts surrounding an individual. It is known that photosynthesis has a relevant impact on changes in the pH of the foraminiferal microenvironment which can affect the composition of the carbonate tests (Lea et al., 1999; Eggins et al., 2004; Russell et al., 2004). However, changes in the pH of the surrounding seawater do not have a significant effect on Mg incorporation in *G. sacculifer* (Dueñas-Bohórquez et al., 2009). According to De Nooijer et al. (2009) and Bentov et al. (2009), the significant increase in pH observed at the site of calcification (pH $\geqslant$ 9) is entirely controlled by the foraminifer itself; while photosynthesis by symbionts may help to increase the already high pH of the calcifying fluid (Zeebe and Sanyal, 2002). Therefore, slight changes in the biological process controlling the pH increase between specimens might be responsible for the observed interindividual variability in Mg/Ca ratios (Fig. 3a).

Cryptic speciation, which refers to individuals that belong to genetically different species but are morphologically identical, can be discarded as a source of interindividual variation in *G. sacculifer*. Darling et al. (1999), Kucera and Darling (2002) and, Darling and Wade (2008) showed that only a single genotype of this species has been identified in the Atlantic and Indo-Pacific Oceans. Alternatively, genetic differences may cause slight differences in the calcification process (i.e. different rates of seawater uptake, Ca-pumping capacity, etc.) that cause the element composition of the calcite to vary between specimens.

4.1.2. Implications of Mg/Ca interindividual variability for the reconstruction of seasonality

Sadekov et al. (2008) suggested that the interindividual variability found in Mg/Ca ratios of final chambers of *Globigerinoides ruber* from core top and plankton tow samples contributes $\sim 1.6 \pm 0.3$ to $\sim 2.5 \pm 0.3$ °C to the temperature variance. This value already excludes the interindividual variation due to seasonality in SST. The interindividual variability found in *G. sacculifer* contributes 2.5 ± 0.5 °C to the apparent temperature variance (standard deviations in Table 5). This implies that the reconstruction of seasonality, based on analysis of single specimens and specific chamber stages, has an inherent inaccuracy of about 2.5 °C.

The temperature variance calculated from the last four ontogenetic stages of *G. sacculifer* is similar to the temperature variance found in *G. ruber* (Sadekov et al., 2008). This suggests that interindividual variability might be similar among planktonic foraminiferal species.

4.2. Interindividual variability in Sr/Ca ratios

The interindividual variability observed in Sr/Ca ratios of *G. sacculifer* is much smaller than the variability observed in Mg/Ca ratios (Table 2 and Fig. 3). Eggins et al. (2003) showed a relatively uniform Sr/Ca ratio within the carbonate test walls of *G. sacculifer*, which implies an absence of Sr/Ca banding. This contributes to more homogeneous Sr/Ca ratios between individuals as it can be observed here (Fig. 3b). Based on this observation, the use of Sr/Ca ratios of *G. sacculifer* as potential proxy for past changes in seawater Sr/Ca (Stoll et al., 1999) and $[CO_3^{2-}]$ (Russell et al., 2004; Dueñas-Bohórquez et al., 2009) is not significantly impacted by variations in Sr-incorporation between specimens.

4.3. Ontogenetic variation of Mg/Ca

The regression model used provides a solid statistical basis to quantify the ontogenetic (chamber stage) effect on Mg/Ca ratios ($p < 0.001$) (Table 3). The resulting Mg/Ca-ontogenetic (chamber) stage relationship indicates that salinity only accounts for 10% of the variation in Mg/Ca ratios ($r^2 = 0.10$; Table 3). From this Mg/Ca-chamber stage relationship, a decrease of 0.43 mmol/mol of Mg/Ca per chamber stage (from the oldest – chamber 17- to the newest-chamber 20) is estimated (Table 3 and Fig. 3a). This result is in agreement with studies by Sadekov et al. (2005) on the same species where older chambers (F-1, F-2 and F-3) are skewed towards higher Mg contents by up to 20–25%. In the present study, chamber stages 19, 18 and 17 (F-1, F-2 and F-3, respectively) are also skewed towards higher Mg/Ca but to a lesser extent (7%). Specimens analysed by Sadekov et al. (2005) were taken from core-top samples; therefore, this high Mg percentage in older ontogenetic

Table 3
Mg/Ca and temperature relationships with ontogenetic (chamber) stage in *G. sacculifer*.[a]

		n	r^2	*F* value	*p*	Experimentally determined responses (in mmol/mol) per chamber stage
Regression	Mg/Ca = 12.42(±2) – 0.43(±0.11) chamber	201	0.10	16.10	<0.001	−0.43
ANOVA	Between chambers	201		7.11	0.001	
Post Hoc Test Hochberg	Chamber 18 vs. Chamber 19	171		–	<0.001	
Regression	Temperature = 47.53(±5) – 1.11(±0.30) chamber	201		16.73	<0.001	−1.11

[a] Temperature and salinity correspond to 26 °C and 36, respectively. Regression and statistics are based on analyses per specimen (not means); "*n*" refers to the number of specimens used to calculate the mean and the standard deviation. Only relationships that are statistically significant ($p < 0.001$; $p < 0.05$) are included, resulting in the exclusion of Sr/Ca vs. chamber-stage; *p* indicates that there is less than a 0.1% or 5% chance that the high F-ratios obtained would happen by chance alone. This means that a regression model overall predicts Mg/Ca variability significantly well under the conditions analysed.

Table 4
Corrected Mg/Ca ratios of GAM and No GAM specimens of *G. sacculifer* from the study by Nürnberg et al (1996a) (data belonging to Table 1 and Fig. 3).

Final chamber type	Average salinity	MgO (ppm)	Mg/Ca (mmol/mol)	Mg-enrichment factor	After ontogenetic correction[a]	After salinity correction[b]	Mg-enrichment factor
GAM	34.25	3404	8.45	**3.30**	7.14	7.03	**2.75**
No GAM	33.25	1033	2.56				

[a] According to equation $Mg/Ca = 12.42(\pm 2) - 0.43(\pm 0.11)$ Chamber (negative correlation) (Fig. 3a). This implies that there's an increase in Mg/Ca from GAM specimens of 1.30 mmol/mol due to an ontogenetic variation of 3 chamber stages (17–20).
[b] According to Dueñas-Bohórquez et al. (2009): $Mg/Ca = 0.11\,S + 1.00$. This implies that there is an increase in Mg/Ca from GAM specimens of 0.11 mmol/mol due to a salinity difference of 1 unit.

stages might well reflect the influence of other parameters rather than an ontogenetic effect alone.

We report a significant effect of ontogeny on Mg/Ca ratios of *G. sacculifer* ($p < 0.001$; Table 3). The Hochberg post hoc test shows that only Mg/Ca ratios from chamber stage 18 are significantly different from the values reported for chamber 19 ($p < 0.001$) (Table 3). The Mg/Ca values measured in chamber stages 17 and 20 were not significantly different from the values reported in the other two chamber (ontogenetic) stages (Fig. 3a). This is probably due to the low number of measurements available for these two chamber stages. Our results show that this ontogenetic effect cannot be due to changes in the calcification temperature related to migration of *G. sacculifer* through the water column. An explanation for the possible origin of this ontogenetic effect is presented in Section 4.5.

On one hand, Nürnberg et al. (1996a) reported significantly higher Mg/Ca ratios in final chambers of specimens of *G. sacculifer* that underwent gametogenesis (GAM). On the other hand Duckworth (1977) reported lower Mg/Ca ratios in the GAM-calcite. Brown and Elderfield (1996) also suggested that the lower Mg/Ca ratios in the keels of planktonic foraminifera are responsible for the slower dissolution of these parts of the tests that the rest of it. Below, we provide three explanations that can reconcile these contrasting results.

Firstly, the cleaning procedures used in our and the cited studies vary: while foraminiferal samples were only rinsed with distilled water before being embedded in resin in the study by Nürnberg et al. (1996a), foraminiferal samples from our study were cleaned in an ultrapure sodium hypochlorite bath (NaClO 5%). The use of NaClO (5%) ensures that the samples are free of organic material which can easily bind to Mg and might be the source of high-Mg around or within the foraminiferal carbonate test walls. Even though the measurements of Mg in the study by Nürnberg et al. (1996a) were performed in the interior of the solid phase (avoiding the calcite surface), the presence of organic material (e.g. rests of cytoplasm), and therefore high Mg layers, cannot be entirely discarded.

Secondly, we cannot be certain that the final chambers from GAM specimens measured in the study by Nürnberg et al. (1996a) correspond to the final chambers from GAM specimens (chamber stages 20 or 19) used in the present manuscript. This is due to lack of information about size (shell diameter) of the specimens in the former study. Therefore, we cannot directly compare our results with those from Nürnberg et al. (1996a). Based on the ontogenetic variation of Mg/Ca found in the present manuscript, we can explain part of the difference found in Mg/Ca between GAM (3403 ppm MgO = 8.44 mmol/mol) and NO GAM (1033 ppm MgO = 2.56 mmol/mol) specimens in the study by Nürnberg et al. (1996a) as follows: the final chambers measured in GAM foraminifera might correspond to a chamber stage 17 or an earlier stage while the final chambers measured in NO GAM specimens could correspond to chamber stages 19, 20 or later stages (slightly bigger specimens). This would result in slightly higher Mg/Ca of GAM specimens.

Finally, GAM specimens analyzed in the study by Nürnberg et al. (1996a) were collected from an average salinity of 34.25 while NO GAM specimens from the same study were grown in an average salinity of 33.25. According to Dueñas-Bohórquez et al. (2009), an increase of 1 salinity unit causes an increase of 0.11 mmol/mol in Mg/Ca; this can also influence the measured Mg/Ca in GAM foraminifera. Based on the previous explanations, we can only explain an enrichment of 0.55 in Mg/Ca of final GAM chambers in the study by Nürnberg et al. (1996a) after considering ontogenetic variation and a salinity effect (Table 4). There is still a considerably high Mg enrichment (2.75) in GAM calcite with respect to NO GAM calcite that could be explained by the presence of Mg-enriched organic matter around or within the calcite.

4.4. Ontogenetic variation of Sr/Ca

No clear ontogenetic effect is observed in Sr/Ca ratios for the last four chamber stages of *G. sacculifer* (Fig. 3b). Our results also show a much smaller interindividual variability for Sr/Ca ratios than for Mg/Ca ratios. Moreover, Mg/Ca and Sr/Ca values from every chamber stage do not correlate ($p > 0.05$; data in EA, Table EA5). This suggests that the mechanism responsible for the partitioning against Mg is at least partly decoupled from the mechanism responsible for Sr incorporation. Therefore, environmental parameters that have a large impact on Mg-incorporation (e.g. seawater temperature) may not affect Sr-incorporation. Additionally, Sr/Ca ratios are known to increase with calcite precipitation rate in inorganic and biogenic calcite (Lorens, 1981; Nehrke et al., 2007; Kısakürek et al., 2008; Tang et al., 2008). The lack of a clear trend in Sr/Ca ratios of *G. sacculifer* suggests that precipitation rates were similar during production of the last four chambers.

Table 5
Temperatures of the chamber stages analysed from *G. sacculifer*.

Chamber stage	Mg/Ca (mmol/mol)	Standard deviation	T_c (°C)[a]	Standard deviation T_c	T_e (°C)[b]	Independent *t*-test (*p*)[c]
17	4.75	1.28	28	2.90	26	0.01
18	4.82	1.09	28	2.60	26	0.04
19	4.13	1.07	26	3.10	26	–
20	3.76	0.63	25	2.10	26	–

[a] T_c corresponds to calculated temperatures using Nürnberg et al. (1996b) equation: $Mg/Ca = 0.39\, e^{0.089T}$ (based only on data from laboratory culture experiments of *G. sacculifer*).
[b] T_e corresponds to expected temperature.
[c] 2-Tailed significance level, *p*. Only values that are statistically significant ($p < 0.05$) are shown. This means that there is a significant difference between the two means compared (data set from Nürnberg et al. (1996b) and data set from every chamber stage).

4.5. Impact of ontogeny on Mg/Ca and Sr/Ca ratios

Sadekov et al. (2005) reported that *G. sacculifer* has bands within its chamber walls with relatively high Mg/Ca ratios, commonly found between thicker layers with lower Mg/Ca ratios. This Mg/Ca-band composition is significantly different between symbiont-bearing and symbiont-barren planktonic foraminifera, the latter having fewer and broader bands with relatively uniform and low Mg/Ca values. This suggests that the presence and abundance of these narrow Mg-enriched calcite bands intercalated between thicker, low-Mg calcite bands might be explained by the activity of symbionts. Accordingly, Eggins et al. (2004) attributed the Mg/Ca banding in *Orbulina universa* to a daily pH change within the calcifying microenvironment, in response to photosynthetic activity of the symbionts during daytime (high pH) and night-time, during which respiration of the symbionts and the host creates a low pH in the surrounding environment (Rink et al., 1998; Wolf-Gladrow et al., 1999).

Variations in pH of the surrounding seawater have an impact on the bulk composition of the carbonate test as it has already been reported for the planktonic foraminifera *O. universa* and *Globigerina bulloides* (Lea et al., 1999; Russell et al., 2004). Thus, the Mg/Ca banding within the walls in these species might be caused by daily changes in the pH of the calcifying microenvironment. However, the Mg/Ca ratios of *G. sacculifer* do not show a clear response to changes in the pH ($[CO_3^{2-}]$) of the surrounding seawater (Dueñas-Bohórquez et al., 2009). Likewise, changes in the pH of the microenvironment may not have any effect on the Mg banding of the test walls.

Previous culture studies with different planktonic species (Lea et al., 1999; Russell et al., 2004; Dueñas-Bohórquez et al., 2009) showed that an increase in pH ($[CO_3^{2-}]$) leads to higher foraminiferal Sr/Ca ratios. According to Dueñas-Bohórquez et al. (2009), a rise of 100 μmol/kg in $[CO_3^{2-}]$ (increasing pH) causes an increase of 0.10 mmol/mol in the Sr/Ca ratio of *G. sacculifer*. If we consider a daily variation of 200 μmol/kg $[CO_3^{2-}]$ in the calcifying microenvironment (photosynthesis vs. respiration; Wolf-Gladrow et al., 1999), this change in $[CO_3^{2-}]$ would result in a Sr banding within the test wall with Sr-enriched layers ~0.2 mmol/mol higher than the low-Sr layers. Contrarily, a number of studies have found Sr to be distributed relatively homogenously in foraminiferal chamber walls (e.g. Eggins et al., 2003; Kunioka et al., 2006). Moreover, we do not observe significant differences in Sr/Ca ratios between chamber stages (Fig. 3b). Therefore, there is evidence to reject the hypothesis of a daily pH change in the microenvironment being responsible for Mg- and Sr-banding within the chamber walls.

Sadekov et al. (2005) showed that the high-Mg/Ca bands are found in all but the last chamber; an observation that can be explained by gametogenesis, where a different calcification mechanism is responsible only for the formation of this chamber (Hamilton et al., 2008). This causes the high-Mg/low-Mg band ratio to vary between chambers resulting in slightly different Mg/Ca values among them: the final chamber has a relatively low Mg/Ca value, whereas previous chambers have higher Mg contents (Fig. 4). Disproportional changes in the thickness of of the high-Mg and low-Mg bands (Sadekov et al., 2005) with further distance (chamber stage 19, 18, 17, etc.) from the new (last) chamber (ontogenetic stage 20) may also contribute to the observed ontogenetic effect found for the four last chamber stages (Figs. 3a and 4). The results of Sadekov et al. (2005) are in agreement with our observations in which older chambers have higher Mg/Ca ratios than new chambers.

According to Erez (2003), the mechanisms involved in chamber formation include the presence of an organic template and the precipitation of $CaCO_3$ on both sides of this organic matrix. Bentov and Erez (2005) showed in the benthic foraminifera *Amphistegina lobifera* that high-Mg calcite (up to 12 mol% Mg) is associated with this organic template. Other studies reported Mg heterogeneity within the test walls of planktonic foraminifera with Mg-enriched calcite layers of up to 1–6 mol% Mg (Eggins et al., 2003; Sadekov et al., 2005). These high-Mg bands can be associated to layers of organic components in the chamber walls (Kunioka et al., 2006), although it is not know to what extent the Mg is directly bound to organic compounds or resides in the calcite facing the organic layers.

The large difference in Mg content of the Mg-enriched layers between benthic and planktonic foraminifera indicates that other mechanisms are also important in determining the average Mg/Ca ratio in foraminiferal calcite (e.g. different Ca^{2+}- and/or Mg^{2+} pumping efficiencies).

Bentov and Erez (2006) also proposed that high-Mg calcite may precipitate from transient amorphous calcite precursors. This transient phase of amorphous $CaCO_3$,

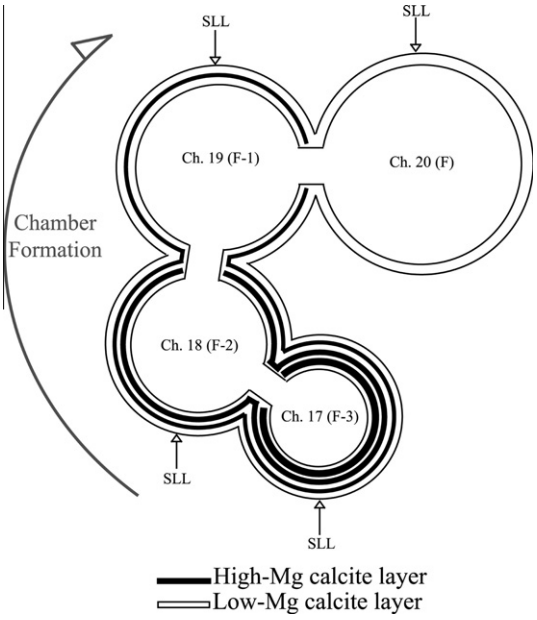

Fig. 4. Possible cause of ontogenetic effect on Mg incorporation: Presence of single low-Mg layer (SLL) in last chambers (Sadekov et al., 2005) and differential change in the thickness of the high-Mg and low-Mg double bands with further distance from the penultimate chamber.

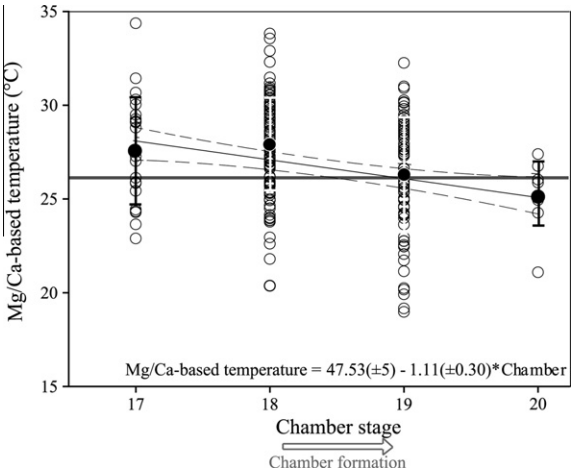

Fig. 5. Temperature values based on measured Mg/Ca ratios from each chamber stage. Mg/Ca-based temperatures were calculated using the following equation: $Mg/Ca = 0.39\ e^{0.089T}$ (based only on data from laboratory culture experiments of *G. sacculifer* in Nürnberg et al., 1996b). Open circles correspond to measurements of individual specimens; closed circles correspond to Mg/Ca average of every chamber stage. Horizontal grey line corresponds to the experimental temperature (26 °C). Error bars indicate ±1 standard deviation. The 95% confidence limits of the curve fit are shown by dashed lines.

which was identified in echinoderms and mollusks (Beniash et al., 1997; Addadi et al., 2003), may have variable trace and minor element concentrations. This might also constitute a source of high-Mg calcite in foraminifera.

Based on our Mg/Ca and Sr/Ca results, the presence of only a low-Mg layer in the final chamber and a disproportional change between the thickness of the high-Mg and the low-Mg calcitic bands is the most suitable mechanism that can explain the ontogenetic effect on Mg incorporation (Fig. 4).

4.6. A size-normalized Mg/Ca-based temperature calibration

Calcification temperatures based on measured Mg/Ca ratios of individual chambers have been calculated using the only available Mg/Ca-temperature calibration for *G. sacculifer* from laboratory cultures: $Mg/Ca = 0.39\ e^{0.089T}$ (Table 5 in Nürnberg et al., 1996b; Fig. 5). This equation is used as it is based only on data from laboratory culture experiments with *G. sacculifer* and thus comparable to the results presented here. The equation derived from the ICP–OES adjustments reported in Nürnberg et al. (2000) was not used here since they included core-top samples of other planktonic species (i.e. *Neogloboquadrina pachyderma* sin.).

The Mg/Ca-temperature calibration proposed here follow the base e-form which was first reported by Lea et al. (1999). This equation is essentially identical to the calibrations reported by Dekens et al. (2002) from core-top samples of *G. sacculifer* ($Mg/Ca = 0.37\ e^{0.09\ T}$), Anand et al. (2003) from sediment-trap samples of 10 planktonic species ($Mg/Ca = 0.38\ e^{0.09\ T}$) and the revised Nürnberg et al. (1996b) equation by Lea (2003) ($Mg/Ca = 0.39\ e^{0.089T}$), which confirms the general applicability of this Mg/Ca-temperature calibration. We find a significant negative correlation ($p < 0.001$; Table 3) between the Mg/Ca based-temperature and chamber stage of *G. sacculifer*, which means there are important variations in the incorporation of Mg into the foraminiferal test among the last four ontogenetic stages of this species. A decrease of ~ 1 °C per consecutive chamber stage (from the oldest to the newest chambers) is reported in the present study (Tables 3 and 4 and Fig. 5).

We observe a significant difference between the Mg/Ca-based temperature (T_{Mg}) means of chamber stages 17 and 18 and the temperature mean predicted by Nürnberg et al. (1996b) ($p < 0.05$; Table 5). On the other hand, no significant difference is observed between the average T_{Mg} of chamber stages 19 and 20 and the temperature average predicted by Nürnberg et al. (1996b) ($p > 0.05$; Table 5). Consequently, temperature calibrations using Mg/Ca ratios from only one ontogenetic stage may result in biased temperature reconstructions. Following the mathematical procedure proposed by Rosenthal and Lohmann (2002), we subsequently changed the pre-exponent constant (B) in order to quantify the ontogenetic effect on Mg incorporation ($Mg/Ca = B\ e^{A\ T}$). Adjusting B to account for ontogeny produces a group of calibrations curves, all with the same temperature dependence ($A = 0.089$; taken from $Mg/Ca = 0.39\ e^{0.089T}$, based only on laboratory culture data of *G. sacculifer*; Table 5 in Nürnberg et al., 1996b) but with varying pre-exponent constants (Fig. 6a). Since both data sets are not affected by post-depositional alterations of Mg/Ca ratio signals, no corrections have to be taken into account. A previously established correlation

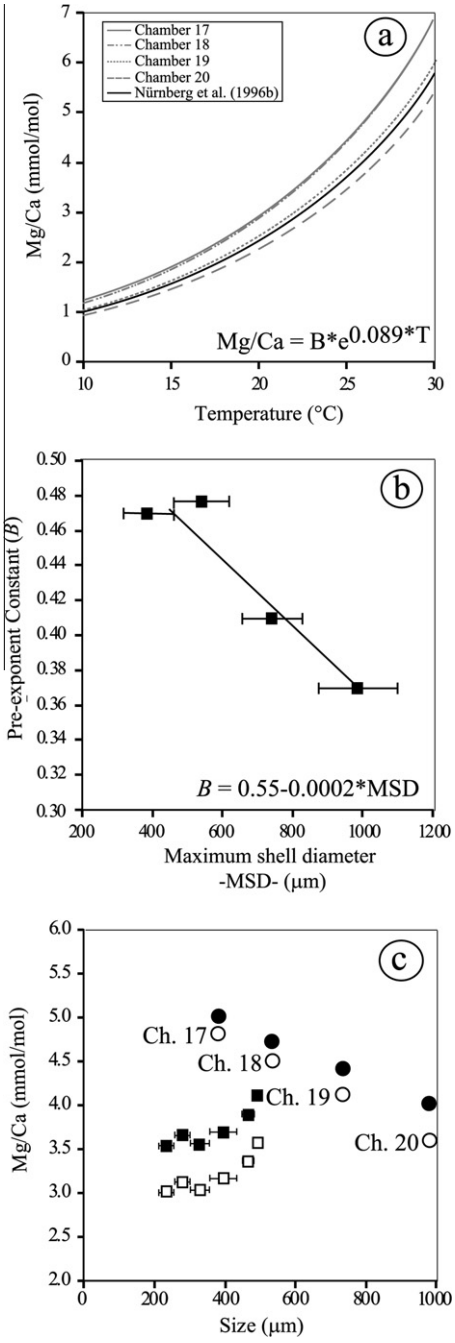

Fig. 6. (a) Mg/Ca–temperature calibrations calculated from the equation $Mg/Ca = B\ e^{0.089T}$ for the last four chamber stages of *G. sacculifer*; (b) Dependence of the pre-exponent constant *B* of every chamber stage on average maximum shell diameter (MSD) (Hemleben and Bijma, 1994); (c) Mg/Ca ratios vs. specimen size; Mg/Ca values per chamber stage correspond to open circles, Mg/Ca for the whole foraminiferal test are represented by closed circles. Open squares correspond to data from Elderfield et al. (2002); closed squares correspond to data from Elderfield et al. (2002) corrected to the temperature used in our study (26 °C), using the Mg/Ca-temperature calibration of Nürnberg et al. (1996b) ($Mg/Ca = 0.39\ e^{0.089T}$).

by Hemleben and Bijma (1994) for *G. sacculifer* was used to convert chamber stages into maximum shell diameter (MSD). Plotting the pre-exponential constant (*B*) versus MSD, we obtain a linear correlation (Fig. 6b). Therefore, a new size corrected temperature equation is proposed for Mg/Ca-thermometry in which the pre-exponent constant is a function of MSD:

$$Mg/Ca_{chamber} = (0.55(\pm 0.03) - 0.0002(\pm 4 \times 10^{-5}) \times MSD)e^{0.089T} \quad (1)$$

This formula is based on the observed change in $Mg/Ca_{chamber}$ ratios in the last four chamber stages (17–20). Therefore, caution must be exerted when applying this equation to earlier ontogenetic stages (i.e. chamber 16, 15, 14, etc.).

The Mg/Ca ratios for all individual chambers were calculated using Eq. (1) (Table 6 and Fig. 6c). These Mg/Ca values were subsequently used to calculate an estimated Mg/Ca ratio for whole specimens. The following mass balance equation was used:

$$\sum_{Ch0-20} (Mg/Ca_{Ch} \times W_{Ch}) = Mg/Ca_T \times W_T \quad (2)$$

where Mg/Ca_{Ch} and W_{Ch} correspond to the Mg/Ca value and the weight of individual chambers, respectively. Mg/Ca_T and W_T refer to the estimated Mg/Ca values and weight for the whole foraminifer, respectively. Size is represented here by the chamber stage number (Ch 0–20). Although this approach requires us to extrapolate Eq. (2) well beyond the calibration interval (last four ontogenetic stages), this is justified by the relative small contribution

Table 6
Estimated Mg/Ca ratios for every chamber stage and whole foraminiferal specimens.

Chamber stage (Ch.st.)	Shell size (µm)[a]	Shell weight (µg)[b]	Mg/Ca per chamber (mmol/mol)[c]	Mg/Ca for whole specimens (mmol/mol)[d]
1	16	0.24	5.53	5.53
2	25	0.38	5.51	5.52
3	28	0.41	5.51	5.52
4	30	0.45	5.50	5.52
5	34	0.51	5.49	5.52
6	37	0.56	5.49	5.52
7	40	0.60	5.48	5.51
8	45	0.68	5.47	5.51
9	52	0.78	5.46	5.50
10	58	0.87	5.45	5.50
11	69	1.04	5.42	5.48
12	75	1.13	5.41	5.48
13	85	1.28	5.39	5.47
14	100	1.51	5.36	5.45
15	175	1.79	5.21	5.41
16	270	3.76	5.02	5.21
17	391	8.88	4.77	4.96
18	545	19.61	4.46	4.68
19	740	40.03	4.07	4.37
20	988	76.95	3.56	3.98

[a,b] Data from Hemleben and Bijma (1994).
[c] Calculated using equation $Mg/Ca = (0.55(\pm 0.03) - 0.0002(\pm 4 \times 10^{-5})\ MSD)\ e^{0.089T}$ at a constant temperature of 26 °C.
[d] Calculated using equation $\Sigma_{Ch\ 0-20}\ (Mg/Ca_{Ch} \times W_{Ch}) = Mg/Ca_T \times W_T$.

of the early test carbonate (the first 16 chamber stages of a specimen with 20 ontogenetic stages, only correspond to 10% of the total weight). Based on this new whole foraminifer Mg/Ca values the pre-exponent constant (B) was fitted again in order to quantify the ontogeny effect for whole foraminiferal tests. The following equation was obtained:

$$Mg/Ca = (0.55(\pm 0.03) - 0.0001(\pm 2 \times 10^{-5})MSD)e^{0.089T} \quad (3)$$

This formula is based on the estimated change in Mg/Ca ratios according to the size of the whole specimen (Fig. 6c).

A more gradual decrease in Mg/Ca values is observed with increasing foraminiferal size when considering whole specimens (Fig. 6c). Elderfield et al. (2002) reported lower Mg/Ca ratios of whole foraminifer test than the Mg/Ca values measured here (Fig. 6c). Since foraminifera in that study were collected from core samples, Mg/Ca ratios had to be normalized to the temperature used in our culture study (26 °C). However, the Mg/Ca ratios of the culture study were still higher compared to the values from the core study after the temperature correction. The lower Mg/Ca ratios in the study by Elderfield et al. (2002) could potentially be due to preferential dissolution of high Mg/Ca phases during alkaline oxidative cleaning. Thus, wet chemical cleaning renders the remains of tests relatively Mg-poor (Haley and Klinkhammer, 2002)

The positive correlation observed between foraminiferal size and Mg/Ca ratios of *G. sacculifer* in core top samples from the Holocene (Elderfield et al., 2002) is opposite to the trend observed in our culture results (Fig. 6c) where physical and chemical parameters of the seawater were kept constant. Therefore, the Mg/Ca-size relationship shown in Elderfield et al. (2002) must be caused by changes in other parameters rather than ontogeny. Seawater temperature has already been ruled out by Elderfield et al. (2002) as the source of Mg/Ca ratio variation with size. When we convert the Holocene Mg/Ca ratios into salinity by using the equation proposed by Dueñas-Bohórquez et al. (2009), we obtain an unrealistic change in SSS of more than 4 within the top 50 m (habitat of *G. sacculifer*). The effect of $[CO_3^{2-}]$ on Mg/Ca ratios has already been analysed by Russell et al. (2004) and Dueñas-Bohórquez et al. (2009), with no significant changes in Mg/Ca within the range of $[CO_3^{2-}]$ values for surface seawater. This indicates that none of these parameters can explain the increase in Mg/Ca with size. Therefore, other parameters (e.g. pressure which causes calcite dissolution), capable of not only masking but overriding the ontogenetic effect, must be responsible for the Mg/Ca-size positive correlation found by Elderfield et al. (2002). Even though these Holocene foraminiferal samples were evaluated for carbonate preservation, partial dissolution may be considered as a possible source of variation in Mg/Ca ratios with size. Rosenthal and Lohmann (2002) use a quantitative method to correct Mg/Ca records for alteration by dissolution and that could potentially be used to determine the dissolution effect on the core-top samples used by Elderfield et al. (2002).

Recently, Hamilton et al. (2008) showed that the planktonic foraminifer *Orbulina universa*, adds a constant amount of calcite, of about 4 μg, to their tests during gametogenesis. When foraminifera add this so-called GAM-calcite deeper in the water column we expect it to be relatively depleted in Mg, because of the lower temperatures. The addition of a constant amount of lower Mg calcite to a variable final test size would result in an apparent increase in Mg/Ca ratios with test size, similar to the trend observed in the field study of Elderfield et al. (2002). In our study, foraminifera were maintained under constant temperature, preventing such an effect. The ontogenetic trend observed in the specimens cultured, lacking this lower-Mg GAM-calcite, is opposite. This implies that the increasing contribution of low Mg/Ca layers during the later life stages of *G. sacculifer* determine the ontogenetic trend. The lack of low-Mg GAM-calcite produced lower in the water column also explains the overall somewhat lower values observed in the data set of Elderfield et al. (2002). This difference decreases as shell size increases as the relative contribution of GAM-calcite becomes less important.

5. CONCLUSIONS

The interindividual Mg/Ca variability found in the planktonic foraminifer *Globigerinoides sacculifer* is an important factor that needs to be accounted for when using this species in paleoceanographic studies. This interindividual variability contributes 2.5 ± 0.5 °C to the apparent temperature variance. Interindividual variability in Sr/Ca ratios is much smaller than the variability found in Mg/Ca ratios. The cause of this difference might be related to the element composition of organic-rich bands present in the test walls of individual foraminifera.

The Mg/Ca ratio decreases by 0.43 mmol/mol per ontogenetic stage (from the oldest to the newest chamber), for at least the last four chamber stages. The ontogenetic effect on Mg/Ca values can be explained by the differential pattern of Mg/Ca banding that constitutes the test walls of the different chamber stages (Sadekov et al., 2005). The Mg/Ca ratio variations due to ontogeny may be explained by the lack of a high Mg layer in the final chamber and the differential change in the thickness of the high-Mg and low-Mg bands with further distance from the newest (last) chamber. There is no ontogenetic effect for Sr incorporation in this species which suggests there are not substantial differences in the growth rates of the last four life stages of *G. sacculifer*. Based on the present results, we confirm the presence of an ontogenetic effect on Mg incorporation that can potentially bias Mg/Ca-based temperature reconstructions. We propose two new empirical Mg/Ca-temperature equation based on Mg/Ca measurements of the last four ontogenetic (chamber) stages and whole foraminiferal test: $Mg/Ca = (0.55(\pm 0.03) - 0.0002(\pm 4 \times 10^{-5})\ MSD)\ e^{0.089T}$ and, $Mg/Ca = (0.55(\pm 0.03) - 0.0001(\pm 2 \times 10^{-5})\ MSD)\ e^{0.089T}$, respectively, where MSD corresponds to the maximum shell diameter (MSD) of the specimen.

ACKNOWLEDGEMENTS

This research was funded by the Dutch Organization for Scientific Research (NWO), under Project No. 855.01.079 (PaleoSalt), the German Research Foundation (DFG) under Grant No. BI 432/4-2 (PaleoSalt) and the European Science Foundation (ESF)

under the EUROCORES Programme EuroCLIMATE through Contract No. ERAS-CT-2003-980409 of the European Commission, DG Research, FP6. The field work was carried out at the marine station of the Department of Marine Sciences, University of Puerto Rico. We thank Howard J. Spero for providing laboratory and field equipment; Bärbel Hönisch, Andrea Lenderink and Milton Carlo for assistance in the field and the collection of planktonic foraminifera. We would like to thank Paul Mason and Gijs Nobbe for assisting with the LA–ICP–MS, Helen de Waard for the ICP–AES analyses, Dineke van de Meent-Olieman for the analyses of DIC samples at Utrecht University and Karoliina A. Koho for helping us with the SEM photos of the planktonic foraminifera. We particularly wish to thank Dr. David Lea and two anonymous reviewers for their critical reviews that improved an earlier version of this manuscript.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.gca.2010.10.006.

REFERENCES

Addadi L., Raz S. and Weiner S. (2003) Taking advantage of disorder: amorphous calcium carbonate and its roles in biomineralization. *Adv. Mater.* **15**, 959–970.

Anand P., Elderfield H. and Conte M. H. (2003) Calibration of Mg/Ca thermometry in planktonic foraminifera from a sediment trap time series. *Paleoceanography* **18**(2), 1050. doi:10.1029/2002PA000846.

Beniash E., Aizenberg J., Addadi L. and Weiner S. (1997) Amorphous calcium carbonate transforms into calcite during sea urchin larval spicule growth. *Proc. R. Soc. London Ser. B* **264**, 461–465.

Bentov S. and Erez J. (2005) Novel observations on biomineralization processes in foraminifera and implications for Mg/Ca ratio in the shells. *Geology* **33**(11), 841–844.

Bentov S. and Erez J. (2006) Impact of biomineralization processes on the Mg content of foraminiferal shells: a biological perspective. *Geochem. Geophys. Geosyst.* **7**(1), 1–11.

Bentov S., Brownlee C. and Erez J. (2009) The role of seawater endocytosis in the biomineralization process in calcareous foraminifera. *PNAS* **106**(51), 21500–21504. doi:10.1073/pnas.0906636106.

Bijma J. and Hemleben C. (1994) Population dynamics of the planktic foraminifer *Globigerinoides sacculifer* (Brady) from the central Red Sea. *Deep-Sea Res.* **41**(3), 485–510.

Bijma J., Faber, Jr., W. W. and Hemleben C. (1990) Temperature and salinity limits for growth and survival of some planktonic foraminifers in laboratory cultures. *J. Foramin. Res.* **20**(2), 95–116.

Brown S. J. and Elderfield H. (1996) Variations in Mg/Ca and Sr/Ca ratios of planktonic foraminifera caused by postdepositional dissolution: evidence of shallow Mg-dependent dissolution. *Paleoceanography* **11**(5), 543–551.

Darling K. F. and Wade C. M. (2008) The genetic diversity of planktic foraminifera and the global distribution of ribosomal RNA genotypes. *Mar. Micropaleontol.* **67**, 216–238.

Darling K. F., Wade C. M., Kroon D., Leigh Brown A. J. and Bijma J. (1999) The diversity and distribution of modern planktic foraminiferal small subunit ribosomal RNA genotypes and their potential as tracers of present and past ocean circulations. *Paloceanography* **14**(1), 3–12.

Dekens P. S., Lea D. W., Pak D. K. and Spero H. J. (2002) Core top calibration of Mg/Ca in tropical foraminifera: refining paleotemperature estimation. *Geochem. Geophys. Geosyst.* **3**(4), 1022. doi:10.1029/2001GC000200.

De Nooijer L. J., Toyofuku T. and Kitazato H. (2009) Foraminifera promote calcification by elevating their intracellular pH. *PNAS* **106**(36), 15374–15378. doi:10.1073/pnas.0904306106.

Dickson A. G. (1990) Thermodynamics of the dissociation of boric acid in synthetic seawater from 273.15 to 318.15 K. *Deep-Sea Res.* **37**, 755–766.

Dickson A. G. and Millero F. J. (1987) A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. *Deep-Sea Res.* **34**, 1733–1743.

Duckworth D. L. (1977) Magnesium concentration in the tests of the planktonic foraminifer *Globorotalia truncatulinoides*. *J. Foramin. Res.* **7**(4), 304–312.

Dueñas-Bohórquez A., da Rocha R. E., Kuroyanagi A., Bijma J. and Reichart G. J. (2009) Effect of salinity and seawater calcite saturation state on Mg and Sr incorporation in cultured planktonic foraminifera. *Mar. Micropaleontol.* **73**, 178–189.

Eggins S., De Deckker P. and Marshall J. (2003) Mg/Ca variation in planktonic foraminifera tests: implications for reconstructing palaeo-seawater temperature and habitat migration. *Earth Planet. Sci. Lett.* **212**(3–4), 291–306.

Eggins S. M., Sadekov A. and De Deckker P. (2004) Modulation and daily banding of Mg/Ca in *Orbulina universa* tests by symbiont photosynthesis and respiration: a complication for seawater thermometry?. *Earth Planet. Sci. Lett.* **225**(3–4) 411–419.

Elderfield H. and Ganssen G. (2000) Past temperature and $\delta^{18}O$ of surface ocean waters inferred from foraminiferal Mg/Ca ratios. *Nature* **405**, 442–445. doi:10.1038/35013033.

Elderfield H., Vautravers M. and Cooper M. (2002) The relationship between shell size and Mg/Ca, Sr/Ca, $\delta^{18}O$, and $\delta^{13}C$ of species of planktonic foraminifera. *Geochem. Geophys. Geosyst.* **3**, 1052–1065.

Erez J. (2003) The source of ions for Biomineralization in Foraminifera and their implications for Paleoceanographic Proxies. *Rev. Mineral. Geochem.* **54**(1), 115–149.

Field A. (2009) *Discovering statistics using SPSS*, third ed. Sage Publications Ltd., 821 pp..

Groeneveld J., Nürnberg D., Tiedemann R., Reichart G., Steph S., Reuning L., Crudeli D. and Mason P. (2008) Foraminiferal Mg/Ca increase in the Caribbean during the Pliocene: Western Atlantic Warm Pool formation, salinity influence, or diagenetic overprint? *Geochem. Geophys. Geosyst.* **9**. doi:10.1029/2006GC001564.

Haley B. A. and Klinkhammer G. P. (2002) Development of a flow-through system for cleaning and dissolving foraminiferal tests. *Chem. Geol.* **185**, 51–69.

Hamilton C. P., Spero H. J., Bijma J. and Lea D. W. (2008) Geochemical investigation of gametogenic calcite addition in the planktonic foraminifera *Orbulina universa*. *Mar. Micropaleontol.* **68**, 256–267.

Hemleben C. and Bijma J. (1994) Foraminiferal population dynamics and stable carbon isotopes. In: *Carbon Cycling in the Glacial Ocean: Constraints on the Ocean's Role in Global Change, NATO ASI Series, Ser. 1: Global Environmental Change 17* (eds. Rainer Zahn et al.). Springer Verlag, pp. 145–166.

Hemleben C., Spindler M., Breitinger I. and Ott R. (1987) Morphological and physiological responses of *Globigerinoides sacculifer* (Brady) under varying laboratory conditions. *Mar. Micropaleontol.* **12**, 305–324.

Kucera M. and Darling K. F. (2002) Cryptic species of planktonic foraminifera: their effect on palaeoceanographic reconstructions. *Phil. Trans. R. Soc. Lond.* **360**, 695–718.

Kunioka D., Shirai K., Takahata N., Sano Y., Toyofuku T. and Ujiie Y. (2006) Microdistribution of Mg/Ca, Sr/Ca, and Ba/Ca ratios in *Pulleniantina obliquilloculata* test by using a Nano-SIMS: implication for the vital effect mechanism. *Geochem. Geophy. Geosyst.* **7**, 12. doi:10.1029/2006GC001280.

Kısakürek B., Eisenhauer A., Böhm F., Garbe-Schönberg D. and Erez J. (2008) Controls on shell Mg/Ca and Sr/Ca in cultured planktonic foraminiferan, *Globigerinoides ruber* (white). *Earth Planet. Sci. Lett.* **273**, 260–269.

Lea D. W. (1999) Trace elements in foraminiferal calcite. In *Modern Foraminifera* (ed. B. K. Sen Gupta). Kluwer Academic Publishers, UK, pp. 259–277.

Lea D. W. (2003) Elemental and isotopic proxies of past ocean temperatures. In *The Oceans and Marine Geochemistry* (ed. H. Elderfield) 6, 365–390. Treatise on Geochemistry (eds. H. D. Holland and K. K. Tuerekian), Elsevier-Pergamon, Oxford.

Lea D. W., Mashiotta T. A. and Spero H. J. (1999) Controls on magnesium and strontium uptake in planktonic foraminifera determined by live culturing. *Geochim. Cosmochim. Acta* **63**(16), 2369–2379.

Lewis E. and Wallace D. W .R. (1998) Program Dev eloped for CO2 System Calculations. ORNL/CDIAC-105. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, Tennessee.

Lorens R. B. (1981) Sr, Cd, Mn and Co distribution coefficients in calcite as a function of calcite precipitation rate. *Geochim. Cosmochim. Acta* **45**(4), 553–561.

Mehrbach C., Culberson C. H., Hawley J. E. and Pytkowicz R. M. (1973) Measurement of the apparent dissociation constant of carbonic acid in seawater at atmospheric pressure. *Limnol. Oceanogr.* **18**, 897–907.

McConnell M. C. and Thunell R. C. (2005) Calibration of the planktonic foraminiferal Mg/Ca paleothermometer: sediment trap results from the Guaymas Basin, Gulf of California. *Paleoceanography* **20**, PA2016. doi:10.1029/2004PA001077.

McKenna V. S. and Prell W. L. (2004) Calibration of the Mg/Ca of *Globorotalia truncatulinoides* (R) for the reconstruction of marine temperature gradients. *Paleoceanography* **19**. doi:10.1029/2000PA000604.

Mortyn P. G., Elderfield H., Anand P. and Greaves M. (2005) An evaluation of controls on planktonic foraminiferal Sr/Ca: Comparison of water column and core-top data from a North Atlantic transect. *Geochem. Geophys. Geosyst.* **6**. doi:10.1029/2005GC001047.

Nehrke G., Reichart G. J., Van Cappellen P., Meile C. and Bijma J. (2007) Dependence of calcite growth rate and Sr partitioning on solution stoichiometry: non-Kossel crystal growth. *Geochim. Cosmochim. Acta* **71**(9), 2240–2249.

Nürnberg D., Bijma J. and Hemleben C. (1996a) Assessing the reliability of magnesium in foraminiferal calcite as a proxy for water mass temperatures. *Geochim. Cosmochim. Acta* **60**(5), 803–814.

Nürnberg D., Bijma J. and Hemleben C. (1996b) Erratum assessing the reliability of magnesium in foraminiferal calcite as a proxy for water mass temperatures. *Geochim. Cosmochim. Acta.* **60**(13), 2483–2484.

Nürnberg D., Müller A. and Schneider R. R. (2000) Paleo-sea surface temperature calculations in the equatorial East Atlantic from Mg/Ca ratios in planktic foraminifera: a comparison to sea surface temperature estimates from $U^{K'}_{37}$, oxygen isotopes, and foraminiferal transfer function. *Paleoceanography* **15**(1), 124–134.

Reichart G., Jorissen F., Anschutz P. and Mason P. R. D. (2003) Single foraminiferal test chemistry records the marine environment. *Geology* **31**(4), 355–358.

Rink S., Kühl M., Bijma J. and Spero H. J. (1998) Microsensor studies of photosynthesis and respiration in the symbiotic foraminifer *Orbulina universa*. *Mar. Biol* **131**, 583–595.

Rosenthal Y. and Lohmann G. P. (2002) Accurate estimation of sea surface temperatures using dissolution-corrected calibrations for Mg/Ca paleothermometry. *Paleoceanography* **17**(3), 1044.

Russell A. D., Hönisch B., Spero H. J. and Lea D. W. (2004) Effects of seawater carbonate ion concentration and temperature on shell U, Mg, and Sr in cultured planktonic foraminifera. *Geochim. Cosmochim. Acta* **68**(21), 4347–4361.

Sadekov A. Y., Eggins S. M. and De Deckker P. (2005) Characterization of Mg/Ca distributions in planktonic foraminifera species by electron microprobe mapping. *Geochem. Geophys. Geosyst.* **6**, 12. doi:10.1029/2005GC000973.

Sadekov A., Eggins S. M., De Deckker P. and Kroon D. (2008) Uncertainties in seawater thermometry deriving from intratest and intertest Mg/Ca variability in *Globigerinoides ruber*. *Paleoceanography* **23**. doi:10.1029/2007PA001452.

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**, 497–500.

Stoll H. M., Schrag D. P. and Clemens S. C. (1999) Are seawater Sr/Ca variations preserved in quaternary foraminifera? *Geochim. Cosmochim. Acta* **63**(21), 3535–3547.

Tang J., Köhler S. J. and Dietzel M. (2008) Sr^{2+}/Ca^{2+} and $^{44}Ca/^{40}Ca$ fractionation during inorganic calcite formation: I. Sr incorporation. *Geochim.Cosmochim. Acta* **72**(15), 3718–3732.

Wolf-Gladrow D. A., Bijma J. and Zeebe R. E. (1999) Model simulation of the carbonate chemistry in the microenvironment of symbiont bearing foraminifera. *Mar. Chem.* **64**(3), 181–198.

Yu J. and Elderfield H. (2008) Mg/Ca in the benthic foraminifera *Cibicidoides wuellerstorfi* and *Cibicidoides mundulus*: temperature versus carbonate ion saturation. *Earth Planet. Sci. Lett.* **276**(1–2), 129–139.

Zeebe R. E. and Sanyal A. (2002) Comparison of two potential strategies of planktonic foraminifera for house building: Mg^{2+} or H^+ removal? *Geochim. Cosmochim. Acta* **66**(7), 1159–1169.

Associate editor: Anders Meibom