Dear Editor,

We thank the editor and two anonymous reviewers for their constructive comments, which helped us to improve the manuscript. Below, we address all comments point-by-point, discussing the subsequent modifications. All suggested changes to wording and punctuation have been incorporated.

On behalf of all co-authors,

Jos Wit

Reviewer #1

As the authors concluded, the species used in this paper was not suitable for a temperature proxy because of its infaunal habit, a weak correlation to temperature and high inter-individual variability. Rather than discussing the application of the Mg/Ca-temperature calibration using this species, the authors should propose criteria to either accept or reject Mg/Ca-temperature calibrations.

We agree with the reviewer that we should propose criteria on which Mg/Ca-temperature calibrations can be evaluated for their usefulness. The criteria on which to accept or reject Mg/Ca-temperature calibrations, however, depend on the desired accuracy and the number of individuals of one species available for analyses. We, therefore, added a paragraph on these criteria and how they vary as well as a table (Table 6) in which the required sensitivity is expressed as a function of uncertainty in reconstructed temperature and number of individuals analyzed.

> "Alternatively, the minimum sensitivity needed to for a certain accuracy in reconstructed temperature can also be expressed as a function of the number of individuals available for analyses of using equation 5 (Table 6). If, for instance, an accuracy of 1 ^{o}C is desirable and there are 20 individuals available for analyses, the sensitivity of the calibration should not be below 0.0738 (equation 5, table 6). This means that a reconstruction based on species such as *B. marginata*, *A. beccarri* and *Uvigerina* spp. will not provide the required precision when 20 or less specimens are analyzed."

N	Uncertainty (°C)					
	2.0	1.5	1.0	0.75	0.50	0.25
10	0.0522	0.0696	0.104	0.139	0.209	0.417
20	0.0369	0.0492	0.0738	0.0984	0.148	0.295
30	0.0301	0.0402	0.0602	0.0803	0.121	0.241
40	0.0261	0.0348	0.0522	0.0696	0.104	0.209

Table 6: Sensitivity (b: equation 1) as a function of a given uncertainty in reconstructed temperature and number of specimens (N) used for Mg/Ca analyses. Uncertainty is expressed as a range (Max-Min) around the average reconstructed temperature

Much of the Discussion has been devoted for sections 4.3 and 5. Most of these sections, however, describe either speculations or hypotheses upon many assumptions without any quantitatively supported data. These sections also seem to be a review paper on the sensitivity of Mg/Ca-temperature calibrations, and are only slightly relevant with the former part of the paper describing the authors' original results. I recommend the authors to prepare another review paper to propose their hypotheses together with many supporting data.

We partially agree with the reviewer and have –also in answer to comments from reviewer #3- deleted part of the Discussion and instead emphasized sections of the Discussion directly related to the Results. We think that sections that explore the relation between sensitivity of Mg/Ca-temperature calibrations, sample size and uncertainty in reconstructed temperatures (based on our own and previously published data) belong in this manuscript to underpin our results and have therefore kept them in the revised version of our manuscript.

In spite of long and thoughtful Discussion, Methods and Results sections are too short and brief to understand in detail. This makes me wonder if presenting the original results is not the main part of this paper.

We present methods and results concisely because most culturing and analytical methods have been presented in a number of previous publications by our group (e.g. Reichart et al., 2003; Dueñas-Bohórquez et al., 2011; Dissard et al., 2010). As a consequence, more space for discussion of (our and previously published) results is available. Mg/Ca-temperature calibrations for many species show a considerable variability in obtained calcitic Mg/Ca (e.g. Nürnberg et al., 1996; Sadekov et al., 2008;

2009; Dueñas-Bohórquez et al., 2011a). Since part of this variability can be caused by environmental variability, general Mg/Ca variability in foraminifera and the biological impact thereon (sections 4.3 and 4.4) is best discussed in combination with results from a culturing experiment, in which environmental change are controlled.

This paper does not answer clearly why inter-individual variability has occurred even if the authors could successfully remove any analytical errors and environmental offsets. Is inter-individual variability after removing the above factors attributed to the vital effect, which differs within individuals of the same species?

This is exactly one of the main aims of our paper. The most important objective of our manuscript is to calculate the magnitude of biologically inherent offsets in foraminiferal Mg/Ca. Without aiming to mechanistically explaining the nature of this variability (the vital effect: see also Bentov and Erez, 2006) we could still show that this variability is present between individuals (Table 2). We now added a paragraph focusing on the relation between the vital effect and inter-individual variability in Mg/Ca.

> "The second source for the large inter-individual variability is the vital effect, caused by variability in the efficiency and rate of various cell-physiological processes that constitute the calcification pathway (Erez, 2003; Bentov and Erez, 2006; De Nooijer et al., 2009a). The impact of these processes can be estimated by correcting the observed Mg/Ca values for the maximum analytical error and the environmentally induced offsets calculated above. If the vital effect would be zero, every measured foraminiferal Mg/Ca value would fit the calibrated regression line (Fig. 5). Although impact of the vital effect cannot be determined directly, we can thus estimate its magnitude."

I wonder bottom water temperature can be reconstructed using infaunal species. Rethink the significance using this species.

The low sensitivity of *B. marginata* limits the use of this species for paleo-temperature calibrations. This is, however, not be the case for all infaunal living foraminifera (e.g. *Pyrgo*; Table 5). Moreover the lack of a carbonate ion effect on infaunal species has been speculated to make them actually more reliable as tracers for deep-water temperatures (e.g. Elderfield et al., 2006). We have added a section on the relation between sample size, accuracy of reconstructed temperature and sensitivity of the Mg/Ca-temperature calibration (see also answer to question #1).

Why did you set this temperature range?

We chose this temperature range (4-14 ^{o}C), because this is the range in which *B. marginata* is found to live in the Bay of Biscay, which was the source area for the used individuals in the culture set-up. This has been added to the methods section of the revised manuscript.

> "Isolated specimens were placed in culture set-ups between 4 and 14 ^{o}C (natural range for *B. marginata* in the Bay of Biscay) at Utrecht University and the University of Angers (8 experiments, table 1)."

Were specimens cultured in Calcein-seawater or in seawater after staining in Calcein-seawater?

Specimens were cultured in seawater after staining in Calcein-seawater.

> "Growth was monitored through incorporation of the fluorescent marker Calcein after which the foraminifera were introduced to the experimental set-up."

Explain how to control water temperature. This is important information for your experiments.

Temperature was controlled in incubators and is added to the methods section. Variability in air-temperature over the course of each experiment is given in table 1 and is over-estimated due to the dampening effect of the seawater in which the foraminifera are cultured.

Alkalinity means Total Alkalinity? Why pH and DIC are connected with slash (/)?
pH and DIC are different parameters.

Alkalinity is indeed total alkalinity unless stated otherwise. The dash between pH and DIC is because in the experiments based at the Utrecht University the DIC was measured, while at the University of Angers the pH was monitored. This has now been clarified in the methods section.

Explain which part of a chamber you measured. Information on the depth and width of measurements are necessary as well. I prefer these are shown in SEM photographs. Assess the influence of lamellar structure (primary and secondary calcite) and organic linings/membranes on measurement data.

Due to the diameter of the ablation crater (80 μm), a relatively large part of the exposed side of each chamber is measured. Depth of each crater pit is equal to the thickness of the ablated chamber wall and therefore varies from chamber to chamber (i.e. chambers built later in life have thinnest walls). Therefore, test wall thickness varies in our dataset and is estimated from the length of an ablation profile. Since this is an indirect measure of test wall thickness we refrained from adding this to the manuscript. Laser ablation Mg/Ca profiles were relatively homogeneous as shown in Figure 1, which excludes the impact of lamellar structures for this species.

I prefer to describe it even if a previous paper described it in detail.

A more detailed description on the Laser Ablation methods is now added to the manuscript.

"Elements were measured on newly calcified chambers of adult foraminifera, as a ratio to calcium with laser ablation inductively coupled mass spectrometry (LA-ICP-MS), using a deep ultraviolet wave length laser (193 nm) with a Lambda Physik excimer laser system with Geolas 200Q optics and a quadrapole ICP-MS instrument (Micromass Platform) (Reichart et al., 2003). Laser ablation spot size was 80 μm and foraminiferal chambers were ablated through the whole outer test wall. Measured elements included ^{24}Mg, ^{26}Mg, ^{27}Al, ^{42}Ca, ^{43}Ca, ^{44}Ca, ^{55}Mn, ^{88}Sr and their relative natural abundances. Mg/Ca ratios were determined using obtained ^{24}Mg concentration and assuming 40 wt% ^{44}Ca in $CaCO_3$. Counts for ^{26}Mg were used to check for consistency of the ^{24}Mg concentrations. Element/Ca ratios were calibrated against the NIST 610 and an in-house calcite standard, verifying that differences in ablation energy do not affect measured elemental concentrations (Hathorne et al., 2008, Wit et al., 2010)."

Elemental ratios with respect to Ca means (24Mg+26Mg)/(42Ca+43Ca+44Ca)?

Mg/Ca was determined using ^{24}Mg since it is more abundant than ^{26}Mg and thus gives a more accurate Mg-concentration. The minor element was used as a check to confirm the ratio obtained by ^{24}Mg. ^{44}Ca was used as an internal standard (40 wt% in $CaCO_3$) to obtain Mg/Ca values. This is added to the manuscript (see previous comment).

For ontogenetic analysis, the number of chambers is more suitable than test size diameter.

We agree, but the very small size of the earliest chambers of *Bulimina marginata* makes it impossible to accurately determine chamber number in this species. For other benthic genera (e.g. *Ammonia*) it has been shown that there is no ontogenetic (based on chamber number) trend in Mg/Ca and Sr/Ca (De Nooijer et al., under review). We have, however, added the possibility that differences in determination of a foraminifer's size/ ontogenetic stage may influence detection of ontogenetic effects.

> "The very small size of the first chambers makes it impossible to count the chamber number in this species, hampering comparison with previously reported ontogenetic trends (or absence thereof) in element/Ca ratios based on chamber number (Dueñas-Bohórquez et al., 2010; De Nooijer et al., under review)."

Results: Explain each result and graph in more detail.

We have added a more elaborate explanation for each of the results (Tables 2 and 3, Figures 2 and 3).

Section 4.2: How about comparing Mg/Ca values with growth rates of each individual during culture periods?

Unfortunately, we do not have growth rates for our experiments, making it impossible to account for the effect of growth rate on Mg/Ca. In a number of recently conducted experiments (De Nooijer et al., under review; Funcke et al., in prep.) we show that growth rates in cultured benthic foraminifera have no effect on Mg/Ca and Sr/Ca. This is, however, outside the scope of the present manuscript.

Average Mg/Ca_{sw} values and standard deviations are assumed based on other experiments measured. Does this not affect your conclusion?

We did not manipulate the elemental composition of the seawater in any of our experiments, ensuring similarity of seawater chemistry between experiments. Therefore, the measured variability in Mg/Ca in a subset of the used seawaters is representative for all experiments.

Which is salinity Eq. (1) ?

This sentence should have stated …salinity (1)… and is hence changed.

This analysis is successful assuming that all calibrations work in the same direction. If this assumption is correct, remaining inter-individual variability shows vital effects, which differ by individuals?

Our results indeed show that the environment-induced changes in foraminiferal Mg/Ca cannot account for all observed variability in Mg/Ca. The remaining variability should therefore be attributed to the vital effect, manifest at the inter-individual level.

L21-24: I have no idea if this assumption is reasonable or not.

We added a number of references on inter-individual variability in a number of foraminiferal species.

> "Average variability (standard deviation) within one temperature experiment for a population of *B. marginata* is 16.3 % (Table 2), which is similar to percentages found in other foraminiferal species (Sadekov et al., 2008; Dissard et al., 2010a; Wit et al., 2010; Dueñas-Bohórquez et al., 2011a)."

Hard to understand how to derive this equation.

The text on the derivation of equation 3 and 4 is now more elaborate and additionally table 4 was added.

Species	Sensitivity	N			
		10	20	30	40
B. marginata [1]	0.045	2.29	1.62	1.32	1.14
A. beccarrii [2]	0.053	1.95	1.38	1.12	0.97
Uvigerina spp. [3]	0.053	1.95	1.38	1.12	0.97
O. umbonatus [4]	0.090	1.15	0.81	0.66	0.57
G. ruber [5]	0.100	1.03	0.73	0.60	0.52
Cibicidoides spp. [6]	0.109	0.95	0.67	0.55	0.47
H. baltica [7]	0.123	0.84	0.59	0.48	0.42
Pyrgo spp. [8]	0.160	0.64	0.46	0.37	0.32

Table 4: The uncertainty in temperature (^{o}C) as a temperature range ($T_{max} - T_{min}$) based on the averaged standard deviation (16.3%) of the culture experiments. $T_{max/min}$ is based on the Mg/Ca value at a temperature plus or minor the uncertainty ($\sigma/\sqrt{n}$). Mg/Ca values are calculated using the species

specific calibrations available for each species of foraminifera 1) This Study 2) Toyofuku et al. (2011) 3) Elderfield et al. (2006) 4) Rathmann et al. (2004) 5) Anand et al. (2003) 6) Lear et al. (2002) 7) Rosenthal et al. (2011) 8) Wit et al. (under review).

Section 5: Does this section not included in the Discussion?

Format of papers in Biogeosciences warrants conclusions to be in a separate section.

Based on a general biological knowledge of foraminifers, three groups with different sensitivities may be related to microhabitats and higher taxonomic groups. Infaunal species belong to a low sensitivity group, epifaunal and planktonic species belong to an intermediate sensitivity group, and porcelaneous species belong to a high sensitivity group. Off course these relationships do not imply any causality at all. However, infaunal species may become evolved to be poorly sensitive to temperature irrelative to phylogeny because of a relatively small variability of temperature within sediments compared to that at sediment surfaces. Alternatively, comparisons of growth rates among these groups may give some clues. Anyway, more supported and quantitative data are necessary to insist your speculations.

We agree with the reviewer and removed the sections from the discussion accordingly.

Fig. 1: How do you think peaks at the start and end? Are these included in the average value?

Parts of the profiles with elevated magnesium at the outer and inner surface of the chamber walls are removed before calculating the average Mg/Ca. This is also added to Figure 1.

Fig. 2: Are average values of 9 and 11 degree C lower than others due to either the small number of data or other reasons?

Variability in the average Mg/Ca is caused by the variability in the Mg/Ca of the individual laser spots. When using a finite number of measurements, this inevitably leads to averages above or below the calculated trendline (in this dataset at 9 and 13 °C). In case of the average Mg/Ca obtained at 9 °C, the deviation may indeed be influenced by the limited number of analysis at this temperature (Table 2).

Fig. 4: Difficult to understand this graph and relevant paragraph. Explain in more detail. Show references for the original data in the caption.

References to the sensitivities used in this figure are added to the caption, as well as more clear explanation on the data depicted in this figure (see also Tables 4, 5 and 6).

Reviewer #3

Provide a brief overview on the environmental controls on foram shell Mg/Ca, i.e., primarily temperature, but also salinity, carbonate system parameters, and seawater-Mg/Ca. It is the multitude of environmental factors influencing Mg/Ca that makes this culturing experiment under controlled conditions so important.

A brief overview of the environmental parameters impacting foraminiferal Mg/Ca values are now included in the introduction.

> "The incorporation of Mg into foraminiferal calcite is thus expected to primarily depend on changes in environmental temperature. However, incorporation of Mg is also affected by other environmental parameters, including salinity, carbonate ion concentration (CO_3^{2-}) and seawater Mg/Ca (Mg/Ca_{SW}) (Nürnberg et al., 1996; Elderfield et al., 2006; Wit et al., under review)."

Provide a more extensive overview of the literature where metabolic effects have been suggested to influence Mg/Ca. How is the metabolic effect expressed, and how is it distinguished from environmental forcing? This includes between-species differences, ontogenetic trends, between-shell, and between-chamber variability. Give a brief explanation on the proposed mechanism, but more concrete than what is stated in lines 4-6, p. 4955.

A more extensive overview of the vital effect is given the introduction.

> "Moreover, most foraminiferal species produce calcite with a Mg/Ca value approximately an order of magnitude lower than those from inorganic precipitation experiments (Bentov and Erez, 2006, Morse et al., 2007). This shows that, besides an environmental control, there is also a strong biological control on Mg incorporation. The difference in element (and isotope) composition between biologically and inorganically precipitated calcium carbonate is often abbreviated as the vital effect (Urey et al., 1951; Weiner and Dove, 2003) and is caused by biological impacts on the calcification process (Erez, 2003; Bentov and Erez, 2006; De Nooijer et al., 2009a). These include modifications of the internal pH, thereby affecting the carbonate ion concentration of the calcification environment, potentially altering the Mg/Ca of the calcite precipitated (Elderfield et al., 2006; Bentov and Erez, 2006; De Nooijer et al., 2009b). Active discrimination against magnesium during production of a privileged space in which high concentrations of Ca^{2+} are actively maintained is another example of how these vital effects impact foraminiferal Mg/Ca values

(Erez, 2003; Bentov Erez, 2006; De Nooijer et al., 2009a)."

Based on the above review a clear definition of the methabolic effect is required for the porpose of this study.

A definition of the vital effect has been introduced in the introduction.

> "The difference in element (and isotope) composition between biologically and inorganically precipitated calcium carbonate is often abbreviated as the vital effect (Urey et al., 1951; Weiner and Dove, 2003) and is caused by biological impacts on the calcification process (Erez, 2003; Bentov and Erez, 2006; De Nooijer et al., 2009a)."

Special attention might be given to endobenthic forams. When migrating through the sediment the ambient pore water chemistry may change, particularly in organicrich sediments where the pH close to the sediment-bottom water interface can be quite acidic. This leads to the question whether (in forams from sediment samples) between-chamber and between-specimens differences in Mg/Ca are due to metabolic effects or whether they reflect the changing micro-environment. Again, this is why this culturing experiment is needed.

A sentence on the inter- and intra-individual variability as a result from either a vital effect or changing chemical gradients in de sediment is added to the introduction.

> "Variability in Mg/Ca between individual tests of the same species, furthermore, suggests that the biologically-induced offset might not be constant within one species. Part of such variability may be caused by changes in the (micro)-environment in which foraminifera calcify. Infaunal benthic species, for example, experience rapidly changing chemical gradients in the sediment that can affect Mg incorporation and introduce intra- and inter-individual variability in Mg/Ca."

Explain why it is important to quantify the metabolic effect on Mg/Ca. How much does it potentially bias paleo-reconstructions? This topic forms a major part of the Discussion and is the primary motivation for this study, so more details are required here.

An explanation on the importance of quantifying the vital effect in Mg/Ca paleo-thermometry is added to the introduction.

"The inter-individual variability as a result of vital effects is significantly affecting temperature reconstructions based on foraminiferal Mg/Ca values (Sadekov et al., 2008; Hathorne et al., 2009). The ability to quantify and recognize the amplitude, and changes therein, of the vital effect is thus of vital importance in improving the accuracy of the Mg/Ca-thermometer."

Explain how the experiment presented here addresses the above questions.

This has been added to the introduction.

"Within the culture setup, maintained at a range of set temperatures, all other parameters influencing foraminiferal Mg/Ca values (salinity, carbonate ion concentration, seawater Mg/Ca) were kept constant in a controlled environment. Such a culturing approach in which environmental parameters are rigorously constant for all individuals is vital for the assessment of intra-individual variability in foraminiferal Mg/Ca due to biologically factors as benthic foraminifera, especially infaunal living species such as *B. marginata*, calcify in a wide range of biogeochemically different micro-environment. This study thus allows accurate quantification of inter- and intra-individual variability as a result of biologically controlled changes in the foraminiferal calcification process, as all other parameters are kept constant within the experiment. This calibration study thus provides insight into the environmental and biological factors potentially offsetting Mg/Ca-temperature calibrations and the effect on the accuracy of this paleo-thermometer."

*Eqs. 4 and 5: Reword using short and clear sentences. Provide a step-by-step explanation of how Eqs. 3-5 were derived, either in this paragraph or in the appendix. Where does the factor of 0.33 come from? 2*16.3% as suggested in the caption of Table 4? The fundamental principle is that Mg/Ca is exponentially dependent on temperature. This means at a given sensitivity a relative (%) uncertainty in Mg/Ca will always translate to the same absolute (_C) uncertainty in T. This should be mentioned. Clarify that "sensitivity" is the exponential constant of the Mg/Ca versus temperature calibration.*

The derivation of the equation has been clarified and table is added to more clarify the origin of all equations (see response to reviewer 1).

"This results in a temperature uncertainty (range of $(Mg/Ca + \sigma/\sqrt{n}) - (Mg/Ca - \sigma/\sqrt{n})$ and thus expressed as a relative % of measured Mg/Ca), which thus independent of the absolute temperature due to the exponential relation between Mg/Ca and temperature, but is

depending on the number of foraminiferal specimens analyzed (N) (Table 4). The relation between temperature uncertainty and sensitivity can now be calculated for each number of specimens analyzed (N) based on table 4 (equation 3)."

"With this equation, the number of specimens that need to be measured for a certain temperature uncertainty as a function of the sensitivity (exponential constant) of the used Mg/Ca-temperature calibration (Figure 6) can be determined. The value of 0.33 is in fact a doubling of the determined relative standard deviation used to calculate the temperature uncertainties."

Species	Sensitivity	N			
		10	20	30	40
B. marginata [1]	0.045	2.29	1.62	1.32	1.14
A. beccarrii [2]	0.053	1.95	1.38	1.12	0.97
Uvigerina spp. [3]	0.053	1.95	1.38	1.12	0.97
O. umbonatus [4]	0.090	1.15	0.81	0.66	0.57
G. ruber [5]	0.100	1.03	0.73	0.60	0.52
Cibicidoides spp. [6]	0.109	0.95	0.67	0.55	0.47
H. baltica [7]	0.123	0.84	0.59	0.48	0.42
Pyrgo spp. [8]	0.160	0.64	0.46	0.37	0.32

*Equation 5, Table 4, Figure 6 and L. 14 of the text are inconsistent. Using the sensitivity from Eq. 2 (0.045) and 94 specimens Eq. 5 yields an uncertainty of 0.76 _C instead of 1.0 _C as written in the text. 0.33/(SQRT(94)*0.045) = 0.76. This value is in line with Table 4, but not with Fig. 6 – the latter indicates a value of 1 (for B. marginata), consistent with the text, but not with the table.*

It should indeed be 0.75 as stated in table 5. This has been changed in the text. Reading the exact value from figure 6 is difficult but value color is between the clear green and blue.

"species specific" implies the comparison of multiple species, but only Bulimina marginata is investigated here. "uncertainty" – of what?

Title has been adjusted accordingly.

"A reappraisal of the vital effect on Mg/Ca values in cultured benthic foraminifer *Bulimina marginata*: assessing temperature uncertainty relationships"

What is the diameter of the laser beam, and how many spot measurements have been carried out for each foram chamber?

The diameter of the laser is 80 um and each chamber is hit once, due to this spot size. This information is added to the manuscript.

"Laser ablation spot size was 80 μm and foraminiferal chambers were ablated through the whole outer test wall."

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