Geochemical and Biological Impacts on Trace and Minor Element Incorporation in Foraminiferal Test Carbonate

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Geochemical and biological impacts on trace and minor element incorporation in foraminiferal test carbonate

Geochemische en biologische invloeden op de inbouw van sporenelementen in het carbonaat van foraminiferen schelpen

(met een samenvatting in het Nederlands)

Efectos geoquímicos y biológicos en la incorporación de elementos traza y menores en carbonatos de foraminíferos

(con resumen en español)

Proefschrift

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door

Adriana Dueñas Bohórquez

geboren op 27 december 1975
te Bogotá D.C., Colombia
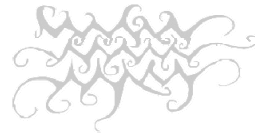
Primero estaba el mar. Todo estaba oscuro. No había sol ni luna ni gente ni animales ni plantas. El mar estaba en todas partes… el mar era la Madre, ella no era gente ni cosa alguna. Ella era espíritu de lo que iba a venir. Ella era pensamiento y memoria.
Ella era Aluna

Mitología Kogui

A Hein

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Chapter 1

General introduction

Since the beginning of the industrial revolution, massive release of CO_2 has affected both global climate and ocean chemistry. To predict future impacts, mankind relies on numerical modeling of the complex Earth system with all its internal feedbacks. To test whether such models reliably describe climate and ocean change as a function of increasing atmospheric pCO_2, they are verified using reconstructions of past natural change. These reconstructions rely on so-called proxies, which relate measurable variables in the fossil record to physical and chemical environmental parameters. From these proxies, the incorporation of trace metals in biogenic carbonate is one of the most commonly used.

The incorporation of minor and trace elements in biogenic carbonate, e.g. tests of foraminifera, reflects parameters such as sea surface temperature and ocean chemistry. Although the incorporation of minor and trace elements into foraminiferal test constitutes an important tool for the reconstruction of past oceans, many aspects involved in the actual uptake of these elements are unknown. Controlled growth experiments and detailed analyses of living specimens, collected from their natural environment, can be used to unravel the processes involved in foraminiferal biomineralization and the effects of physical and chemical ocean parameters on the chemistry of foraminiferal test.

Foraminifera are single-celled amoeboid organisms that belong to the Kindom Protista, Phylum Sarcomastigophora, Class Granulo-reticulosea. However, modern taxonomists rank the group as a phylum. The main characteristics of this taxon are the presence of a highly organized cytoplasm with a net-like array of pseudopods and granular rhizopodia and, the presence of a test that, depending on the foraminiferal group, can be made of biogenic calcium carbonate (calcareous), sediment particles (agglutinated) or organic compounds (polysaccharides).

Foraminifera are abundant in both marine sediments (benthic) and water column (planktonic). The former are present since the early Cambrian (around 525 Ma) while their planktonic counterparts were first found approximately 200 Ma ago. Since then, both types of foraminifera have been present in the fossil record, presently accounting for more than 10.000 living species, of which approximately 40 correspond to planktonic forms. Their worldwide distribution, preservation in the fossil record, and presence in most parts of the geological history of the planet, make foraminifera one of the most commonly used tools in paleoceanography and paleoclimatology.

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For more than 50 years the test composition of fossil foraminifera has routinely been used to reconstruct past climate. Initially, these studies were based on the stable isotopic composition of the tests (Emiliani, 1955); later, the trace and minor elemental composition was also measured and used for proxy-based reconstructions (Boyle, 1981; Graham et al., 1982). Today, by combining foraminiferal stable isotopes and trace and minor elements, variations in the chemistry, temperature and salinity of the ocean can be more accurately reconstructed (Mashiotta et al., 1999; Elderfield and Ganssen, 2000).

Challenges associated with foraminiferal proxies: Part I

Several types of foraminiferal proxies have been identified and are commonly used in paleoceanographic research: physical and chemical proxies (e.g. Mg, Sr, δ^{11}B, δ^{18}O), nutrient and productivity proxies (e.g. Ba, Cd, δ^{13}C), and proxies for early diagenesis (e.g. Mn). However, during the last decade it has become clear that foraminiferal proxies are often affected by more than one ocean parameter at the time, making their interpretation more challenging (Nürnberg et al., 1996a and b; Lea et al., 1999; Ferguson et al., 2008).

Incorporation of Mg into foraminiferal calcitic tests is frequently used for reconstructing sea surface temperature (Rosenthal et al., 1997) and, together with foraminiferal stable oxygen isotope ratios, can also be used to reconstruct salinity (Rohling, 2000). Similarly, variations in foraminiferal Sr incorporation are partly attributed to past changes in seawater Sr/Ca ratios and partly to changes in pCO$_2$ (Stoll et al., 1999). However, recent studies show that other seawater parameters such as salinity and carbonate chemistry, might have an additional impact on Mg and Sr incorporation, that can offset the established calibrations (Russell et al., 2004; Ferguson et al., 2008). Consequently, the goal of the first two chapters of this thesis is to determine the independent impact of the most relevant seawater carbonate parameters on the incorporation of these frequently used proxies.

The effect of salinity on Mg and Sr incorporation in the planktonic foraminifera *Globigerinoides sacculifer* (*sensu stricto*) that were grown in the laboratory under different environmental conditions is quantified in **Chapter 2**. This planktonic species was selected as it is a surface mixed-layer dwelling species, widely used for sea surface temperature

reconstructions. Laboratory experiments allowed to separate a direct salinity effect from a possible independent impact of the calcite saturation state of the seawater (Ω). Although the temperature effect is more important than the salinity effect, a change of 4 salinity units is equivalent to a 1°C bias on Mg/Ca-based temperatures. This effect of salinity on Mg incorporation is minor. However, when using Mg/Ca-based temperatures in combination with foraminiferal $\delta^{18}O$ to calculate salinity, it cannot be neglected. This study also shows salinity as the overriding control on Mg incorporation within the range of Ω studied (Ω between 5.25 and 6.50; $[CO_3^{2-}]$ between 218 and 270 µmol/kg) at a constant temperature of 26 °C. In contrast, Ω appears to be the main control on foraminiferal Sr incorporation (0.10 mmol/mol per 100 µmol/kg rise in $[CO_3^{2-}]$), whereas salinity has a non-significant influence on the incorporation of this trace element.

The calcite saturation state of the seawater, Ω, controls the formation and dissolution of calcite ($CaCO_3$); thus it plays a major role in the global carbon cycle. It is also a function of the calcium and carbonate concentrations of seawater ($[Ca^{2+}]$ and $[CO_3^{2-}]$, respectively). Apart from the effect of the $[CO_3^{2-}]$, and therefore Ω, on the biology of foraminifera, its consequences for the paleoceanographic use of foraminiferal Mg/Ca and Sr/Ca ratios has been a key issue in several studies (Lea et al., 1999; Russell et al., 2004; Mortyn et al., 2005; Rosenthal et al., 2006; Elderfield et al., 2006). In order to separate the effect of Ω from $[Ca^{2+}]$, $[CO_3^{2-}]$ and the other carbonate parameters of the seawater, laboratory culture experiments were carried out using two species of benthic foraminifera.

In **Chapter 3**, the effect of $[Ca^{2+}]$ in seawater and thereby the calcite saturation state (Ω), on Mg and Sr incorporation in benthic foraminiferal calcite was determined. For this set of experiments, individuals of the shallow-water species *Heterostegina depressa* (high-Mg calcite, symbiont-bearing) and *Ammonia tepida* (low-Mg calcite, symbiont-barren) were cultured under a range of different $[Ca^{2+}]$ but similar Mg/Ca ratios. Results of this chapter are discussed in terms of partition coefficients, D_{ME} ($D_{ME}=(ME/Ca_{calcite})/(ME/Ca_{seawater})$; where ME = minor elements) in order to eliminate the impact of slightly different Mg/Ca ratios in the solutions used. The partition coefficient of Mg (D_{Mg}) in *A. tepida* significantly decreases with increasing $[Ca^{2+}]$ and, therefore Ω, while D_{Sr} in the same benthic species does not vary. Conversely, D_{Mg} of *H. depressa* shows only a minor decrease with increasing $[Ca^{2+}]$ and thus Ω , while D_{Sr} increases considerably with $[Ca^{2+}]$ (and also Ω). The different

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responses to seawater chemistry of the two species may be explained by a difference in the calcification pathway that is, at the same time, responsible for the contrasting Mg incorporation between these two species. The impact of Mg incorporation on seawater $[Ca^{2+}]$ in low-Mg species shows the need to correct for changes in calcium concentration in order to reconstruct reliable temperatures from fossil Mg/Ca ratios.

In **Chapter 4**, in contrast to the previous chapter, the effect of the calcite saturation state (Ω) on Mg and Sr incorporation is determined in terms of the carbonate ion concentration of the seawater ($[CO_3^{2-}]$). Two species of shallow-water benthic foraminifera were selected: *Ammonia tepida* and *Heterostegina depressa*. An increase in the $[CO_3^{2-}]$ of seawater and thus Ω, slightly promotes the incorporation of Mg and Sr into the calcitic tests of these species. The response of Mg and Sr incorporation in both species is comparable, even though the total Mg/Ca ratio is ~70 times higher in *H. depressa* than in *A. tepida*. Thus, we show that with increasing $[CO_3^{2-}]$, calcification processes are affected in similar ways, although the magnitude of this effect varies between species. The $[CO_3^{2-}]$ has a minor and opposite influence on Mg incorporation compared to the effect of $[Ca^{2+}]$ (in chapter 3), even though they both affect the calcite saturation state of the seawater (Ω). This implies that Ω is not directly responsible for the altered Mg incorporation into the calcite of *A. tepida* and *H. depressa.* The combined results of these two studies indicate that ions (Ca^{2+} and CO_3^{2-}) involved in biomineralization are taken up through separate transport mechanisms. The similar response of Mg and Sr incorporation in this study suggests that only differences in the Ca^{2+} transport mechanism affect divalent cation uptake. The two ion transport processes are thus apparently independent.

Challenges associated with foraminiferal proxies: Part II

The trace metal and isotope chemistry of foraminiferal tests varies not only as a function of changes in the environment of calcification mentioned above, but also in response to complex physiological processes. The latter is known as the "vital effect", which refers to biologically controlled processes that 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). The goal of **Chapter 5** is to determine the magnitude and mechanisms behind inter-individual and ontogenetic effects on Mg and Sr incorporation in the planktonic foraminifera *Globigerinoides*

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sacculifer, under otherwise controlled physical and chemical seawater conditions. Whereas temperature is the overriding control on Mg/Ca ratios, the inter-individual variability observed in the Mg/Ca values contributes 2 to 3°C to the apparent temperature variance. Inter-individual variability in Sr/Ca ratios is much smaller than that observed in Mg/Ca values. The variability due to ontogeny corresponds to -0.40 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. The presence of a significant ontogenetic effect on Mg incorporation can potentially offset Mg/Ca-based temperature reconstructions. We propose a new empirical Mg/Ca-temperature equation based on calculated Mg/Ca ratios for the whole foraminiferal test: $Mg/Ca = (0.55-0.0001*MSD)e^{0.089 * T}$, where MSD corresponds to the maximum shell diameter of the individual.

Microhabitat preferences of benthic foraminiferal species are probably reflected in their test chemistry, depending on their infaunal and more epifaunal habitat choice. Pore-water chemistry is modified by ongoing early diagenesis and benthic foraminifera that precipitate their tests from pore-water thus reflect the chemical conditions of this surrounding water. In **Chapter 6**, specimens of deep sea benthic foraminifera *Cibicidoides pachyderma* were collected from core-top samples from the Bay of Biscay and the chemical composition of their test calcite was analysed.

Overall composition of the tests is in line with earlier studies, even though the Mg concentration is appreciably lower. Inter and intra-individual variations of the minor and trace element composition of the tests reflect changes in chemistry of bottom and pore-water. The Ba and Mn incorporation into the calcite test indicates vertical migration within the top few centimetres of the sediment. The lack of inter and intra-individual variation in the Sr/Ca ratios indicates that Sr is apparently not affected by either pore-water chemistry, migration patterns or changes in vital effects. The intra-individual variability in Mg/Ca ratios cannot be explained by migration alone, whereas changes in Ba and Mn concentrations in the pore-water are more directly reflected in the test carbonate.

A possible explanation for the observed changes in foraminiferal Mg/Ca ratios involves the presence of food cysts around specimens during

early life stages at the sediment-water interface. The presence of a food cyst influences pH, which is reflected in the foraminiferal Mg incorporation. The Mg/Ca inter-individual variability found in *C. pachyderma* potentially contributes 2°C to the apparent temperature variance.

A brief synopsis of this thesis is given in **Chapter 7**, including implications for biomineralization and future foraminiferal proxy applications.

Chapter 2

Effect of salinity and seawater calcite saturation state on Mg and Sr incorporation in cultured planktonic foraminifera

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J. Bijma, G. J. Reichart

Abstract

Trace elements incorporated in planktonic foraminiferal test carbonate are commonly used as paleoproxies. For instance, Mg/Ca ratios are frequently used for reconstructing sea surface temperature and, together with the foraminiferal stable oxygen isotope ratios, are also used as paleosalinity proxy. Foraminiferal Sr/Ca ratios constitute another example of the application of trace elements in paleostudies since they may reflect the Sr/Ca values of seawater. However, over the past few decades it has been proven that the incorporation of trace elements in foraminiferal calcite is controlled by more than one environmental parameter. To quantify the effect of salinity on Mg and Sr incorporation planktonic foraminifera *Globigerinoides sacculifer* (*sensu stricto*) were grown in the laboratory under different environmental conditions. Laboratory experiments allowed us to separate a direct salinity effect from a possible independent impact through differences in the calcite saturation state of the seawater (Ω). Although the temperature effect is more important than the salinity effect, a change of 4 salinity units is equivalent to a 1°C bias on Mg/Ca-based temperatures. This effect of salinity on Mg incorporation is minor. However, when using Mg/Ca-based temperatures in combination with foraminiferal $\delta^{18}O$ to calculate salinity, it cannot be neglected. The present study shows salinity as the overriding control on Mg incorporation within the range of Ω studied (Ω between 5.25 and 6.50; ($[CO_3^{2-}]$ between 218 and 270 µmol/kg) at a constant temperature of 26°C. In contrast, Ω appears to be the main control on foraminiferal Sr incorporation (0.10 mmol/mol per 100µmol/kg rise in $[CO_3^{2-}]$), whereas salinity has a non significant influence on Sr/Ca.

2.1. Introduction

Over the past two decades temperature reconstructions have become increasingly reliant on Mg/Ca ratios measured on planktonic and benthic foraminiferal shells (Nürnberg et al., 1996a; Lea et al., 1999; Russell et al., 2004; Rosenthal et al., 2006; Yu and Elderfield, 2008). The incorporation of Sr into foraminiferal calcite may also show temperature dependence (Lea et al., 1999; Reichart et al., 2003; Graham et al., 2005). However, other parameters might also be involved in the control of foraminiferal Mg/Ca and Sr/Ca ratios namely pressure (Elderfield et al., 1996), calcite saturation (Lea, 1999), carbonate ion concentration ($[CO_3^{2-}]$) (Mortyn et al., 2005; Rosenthal et al., 2006; Hendry et al., 2009), ontogeny (Ni et al., 2007) and growth rate (Kısakürek et al., 2008). Accurate temperature reconstructions, therefore, rely on an understanding of these additional factors that can potentially influence the incorporation of Mg and Sr in foraminiferal calcite.

Through culture experiments salinity showed to exert a small but consistent influence on foraminiferal Mg/Ca ratios (Nürnberg et al., 1996a; Lea et al., 1999; Kısakürek et al., 2008). More recently, Ferguson et al. (2008) suggested that salinity had a greater than hitherto expected influence on foraminiferal Mg/Ca ratios (15 to 59% increase per salinity unit) using a core-top calibration from the Mediterranean Sea. Such a large salinity dependency would imply that Mg/Ca based temperature reconstructions can be severely biased (e.g. Ferguson et al., 2008; Groeneveld et al., 2008). Foraminiferal Sr/Ca ratios are also affected by salinity, i.e. 4% increase per salinity unit in *Orbulina universa* (Lea et al., 1999). However, it remains unclear whether salinity itself influences foraminiferal incorporation of these two trace elements or whether it is due to changes in the carbonate chemistry associated with the changes in salinity. Since both salinity and total alkalinity (TA) are conservative parameters, by increasing salinity, carbonate parameters, such as TA, dissolved inorganic carbon (DIC) and $[CO_3^{2-}]$ also increase.

Seawater carbonate ion concentration $[CO_3^{2-}]$ has an effect on planktonic and benthic foraminiferal Mg/Ca and Sr/Ca ratios as well (Russell et al., 2004; Mortyn et al., 2005; Elderfield et al., 2006; Rosenthal et al., 2006; Kısakürek et al., 2008; Rathmann and Kuhnert, 2008; Yu and Elderfield, 2008; Raitzsch et al., 2008). When $[CO_3^{2-}]$ of the seawater changes, the calcium carbonate saturation state of seawater (Ω) also changes since $\Omega = [Ca^{2+}]*[CO_3^{2-}]/K_{sp}$, where K_{sp} represents the solubility product at the *in situ* conditions of temperature, salinity and pressure (Zeebe and Wolf-Gladrow, 2005). The carbonate saturation state

might control the rate of calcite precipitation, which in turn is known to influence trace metal incorporation. This varies depending on the particular trace element studied (Lorens, 1981; Nehrke et al., 2007).

Here we present the results of culture experiments in which salinity is kept constant while the $[CO_3^{2-}]$ varies and vice versa. In the marine environment $[CO_3^{2-}]$ covaries with salinity, making it difficult to evaluate these parameters independently. Moreover, since salinity not only affects $[CO_3^{2-}]$, but also $[Ca^{2+}]$, Ω changes quadratically, potentially enhancing its impact. Therefore, the aim of the present culture study is to separate the effects of both salinity and Ω. This way we are able to quantify independently the impact of these two parameters on the incorporation of Mg and Sr into the tests of the planktonic foraminiferal species *Globigerinoides sacculifer* (Brady, 1877).

2.2. Methodology

2.2.1 Foraminifera collection and culturing

Planktonic, symbiont-bearing foraminifera *Globigerinoides sacculifer* (Figure 1A) were collected from surface waters (2-6 m depth), 10 km off the southwest coast of Puerto Rico (17° 54' 46''N, 66° 58' 44''W) by scuba diving 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 foraminifera using an inverted light microscope subsequently transferring the specimens to 120-ml Pyrex bottles with one of two types of filtered seawater namely: 1. Unmodified seawater (controls) or, 2. Seawater whose carbonate chemistry was modified. These bottles were incubated in water baths (0.50 x 1.50 m approx.) and kept at constant temperature (26 °C ± 0.5) and light intensity (12-hr light:12-hr dark cycle using a light intensity of 353 μE/m^2/s). Specimens were fed every third day with a 1-day-old nauplius of *Artemina salina*, starting on the day of collection. For these experiments, we used values of physical and chemical parameters within the tolerance ranges of *G. sacculifer* (Bijma et al., 1990) and followed the procedures outlined in Bijma et al. (1990) in order to minimize stressful conditions that could potentially affect foraminiferal trace element incorporation.

Specimens were monitored daily and usually underwent gametogenesis (GAM) between 7 and 14 days after collection. Then they were removed from the solutions and rinsed with deionised water. We measured individual chambers only from GAM specimens and archived them for later analysis. Foraminifera that built new chambers in the laboratory were identified by comparing their size at collection with the size of the test after gametogenesis. Foraminifera used for trace metal analyses formed 1 to 3 additional chambers during the incubations. Results presented here are based on analysis of the sac-like morphotype (= *G. sacculifer* sensu stricto (Bijma and Hemleben, 1994)).

According to the previously established correlation between chamber number and shell size of *G. sacculifer* (Hemleben and Bijma, 1994), almost all F-1 chambers (= penultimate) correspond to chamber-stage 18 (referred as chambers 18) which corresponds to an average shell size of 545 µm. Consequently, chamber-stage 19 refers in most of the cases to sac-like or F (ultimate) chambers.

Mg/Ca and Sr/Ca values used in this study are only from chambers 18 of *G. sacculifer* sensu stricto (sac-like morphotype) since we observed a more consistent relationship between this chamber stage and the carbonate parameters analysed (salinity, Ω and $[CO_3^{2-}]$) (Tables A3 and A4). Chambers corresponding to other life stages (i.e. 19 and 20) are not taken into account in the present study because ontogeny potentially plays an important role in determining their chemical composition (Nürnberg et al., 1996a; Eggins et al., 2003; Erez, 2003).

Table 1. Overview of experiments with salinity and carbonate saturation state values.

Ω^a / Salinity	30	36	39
5.25± 0.03	Control 1		
6.16 ± 0.02		Control 2	
6.50 ± 0.04			Control 3

[a]Saturation values correspond to the values of the natural salinities (controls).

2.2.2 Carbonate chemistry of the culture solutions

Culture solutions with three different salinity values (30, 36 and 39) were prepared (Table 1). Carbonate parameters of all solutions were calculated using the CO_2sys program (version 01.05, Lewis and Wallace, 1997) and monitored every three days by measuring salinity, total alkalinity, and pH (NBS scale) of the solutions (Table 2A). The corresponding Ω values were:

5.25 ± 0.03, 6.16 ± 0.02, and 6.50 ± 0.04. The majority of present–day surface water conditions fall within the limits of these values (Table 1). In order to obtain the target values (30 and 39), salinity was altered by adding deionised water or through evaporation by sub-boiling (at ~40°C).

In order to reach the target Ω values, we varied the $[CO_3^{2-}]$ of the seawater. Filtered seawater was modified to obtain a range of $[CO_3^{2-}]$ by maintaining a constant dissolved inorganic carbon concentration (DIC) (closed system) and adjusting alkalinity with NaOH (1M) or HCl (1M). Under these conditions, both pH and $[CO_3^{2-}]$ of the seawater increase or decrease with alkalinity. Seawater samples from control experiments (Ω not modified) were collected and brought to the home laboratory at Utrecht University to analyze DIC**.** This carbonate parameter was measured using a Total Organic Carbon Analyzer (Shimadzu, Model TOC-5050A). DIC results from these analyses and DIC values calculated via the CO_2sys program were not significantly different (Table A1).

2.2.3 Sample preparation and analysis

Specimens that grew new chambers were placed in a sodium hypochlorite bath (NaClO 5 %) for 20 minutes in order to remove any remaining organic material. The specimens were rinsed 3 times with deionised water afterward, carefully pipetting of the supernatant. Mg/Ca ratios were measured by laser-ablation inductively coupled plasma-mass spectrometry (LA-ICP-MS, Micromass Platform). This technique allowed us to measure the trace elemental concentrations of individual chambers from single specimens several times.

Table 2. A. Experimental conditions[a].

Experiment No.	Salinity	Alkalinity (µmol/kg)	pH (NBS scale)	Mean $[CO_3^{2-}]$ (µmol/kg)	DIC (µmol/kg)	Ωc
1 (Control 1)	30	2001 ± 29	8.29	208 ± 2	1702	5.2
2	36	2358 ± 13	8.17	222 ± 2	2046	5.3
3	39	2519 ± 26	8.10	224 ± 3	2210	5.2
4	30	2072 ± 32	8.37	246	1719	6.2
5 (Control 2)	36	2416	8.24	258	2053	6.2
6	39	2558 ± 38	8.19	264 ± 2	2188	6.1
7	30	2113 ± 34	8.40	262 ± 3	1739	6.6
8	36	2440 ± 37	8.26	270 ± 2	2059	6.5
9 (Control 3)	39	2603 ± 4	8.21	278 ± 2	2216	6.5

Values are given with ± standard deviations
[a]All 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 CO_2sys (Lewis and Wallace, 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.

Table 2.B. Summary of mean Mg/Ca and Sr/Ca ratios from chambers stage No. 18 in cultured planktonic foraminifera.

Experiment No.[a]	n[b]	Mg/Ca (mmol/mol)	n[b]	Sr/Ca (mmol/mol)
1 (Control 1)	3	4.42 ± 1.30	3	1.32 ± 0.05
2	2	4.42 ± 1.01	2	1.30 ± 0.10
3	4	5.61 ± 1.28	4	1.44 ± 0.15
5 (Control 2)	6	5.16 ± 0.63	7	1.35 ± 0.07
6	2	5.86 ± 0.52	2	1.41 ± 0.01
7	2	3.50 ± 0.89	2	1.38 ± 0.04
8	2	4.40 ± 0.34	2	1.32 ± 0.11
9 (Control 3)	5	5.03 ± 1.34	4	1.34 ± 0.10

Values are given with ± standard deviations.
[a]Experiment No. 4 is not reported due to lack of analysis.
[b]n refers to the number of specimens used to calculate the mean and the standard deviation.

Individual foraminiferal chambers were ablated using a 193 nm laser (GeoLas 200Q Excimer) in a helium flushed ablation chamber which was coupled to the ICP-MS. A deep ultra violet-wavelength laser was used in order to guarantee the reproducibility of the ablation of the fragile tests. This type of laser was employed since carbonates do not absorb laser radiation well at higher wavelengths. 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 analyzed with respect to time (Figures 1A and B). Mg/Ca and Sr/Ca ratios correspond to the average value of single measurements during a laser ablation-ICPMS analysis (Figure 1B). Calibration was performed against U.S. National Institute of Standards and Technology (NIST) SRM 610 glass with ^{44}Ca as an internal standard. Using calcium as an internal standard is ideal as this element is present at a constant concentration of 40%. 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 glass standard SRM 610 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 an uncommonly homogeneous calcite crystal (Icelandspar) that was analysed by LA-ICP-MS. Subsamples taken from this calcite crystal were also dissolved in ultra clean HNO$_3$ and subsequently analysed using an ICP-AES
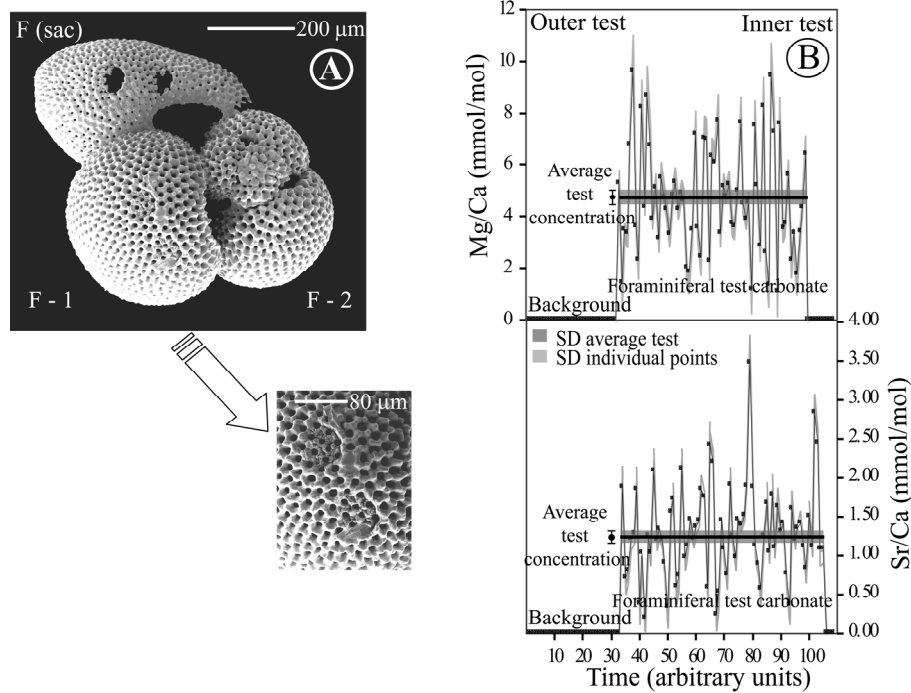
Figure 1. A. Specimen of *Globigerinoides sacculifer* (sac-like morphotype) analysed by Laser ablation-ICP-MS; F (sac) = final chamber or in most of the cases chamber-stage 19; F-1 = penultimate chamber or in most of the cases chamber-stage 18; F-2 = third chamber/ chamber-stage 17 (older than F and F-1) and detail of LA crater; B. Laser ablation profile of foraminiferal calcite.

(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/Ca and Sr/Ca values are statistically identical (Table A2). Based on repetitive analyses of the calcite standard throughout the analytical period, relative precission of the LA-ICP-MS analyses for Mg and Sr was around 3% and 3.6%, respectively. Monitoring simultaneously ^{42}Ca, ^{43}Ca, and ^{44}Ca showed isotopic ratios expected on basis of their natural relative abundances. Normalization to an international standard is also recommended for the analysis of Mg/Ca and Sr/Ca ratios apart from the use of SRM 610 glass (NIST) and homogeneous calcite crystal (Icelandspar) standards. This is suggested in order to avoid methodological variations that could bias the response of these foraminiferal elements to seawater physical and chemical parameters. Accuracy for each individual analysis was calculated using the Glitter computer program, which we 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. An analysis of variance (ANOVA) was used in order to determine how well a regression model would predict Mg/Ca and Sr/Ca variability under the conditions analysed.

2.3. Results

Overall the test carbonate of *Gobigerinoides sacculifer* has lower Mg/Ca ratios (this study; Nürnberg et al., 1996b; Anand et al., 2003) compared to *Orbulina universa* and *Globigerinoides bulloides* (Lea et al., 1999; Elderfield and Ganssen, 2000; McConnell and Thunell, 2005; Russell et al., 2004). Mg/Ca ratios in the tests of *G. sacculifer* and *Globigerinoides ruber* (white) (Kısakürek et al., 2008) show similar values (Figure 2A). Our results correspond to a temperature of 26 °C and variable salinities, ranging from 30 to 39. Mg/Ca ratios measured on chambers 18 of *G. sacculifer* show slightly higher values compared to previous studies (Nürnberg et al., 1996b; Anand et al., 2003) (Figure 2A). However, the combined Mg/Ca values of chambers 18 and 19 correspond well to previous correlations between seawater temperatures and foraminiferal Mg incorporation (Figure 2B). The observed variability between Mg/Ca values of individual specimens grown at constant environmental conditions is larger than the analytical precision of the LA-ICP-MS analyses (Table A4).

2.3.1 Effect of salinity on Mg/Ca and Sr/Ca ratios

We combined the Mg/Ca ratio data of each salinity experiment (30, 36, and 39), regardless of their Ω value (Tables 1 and 2; Figure 3), in order to improve the statistical basis of the Mg/Ca-salinity correlation. The average Mg/Ca values of *G. sacculifer* increase at higher salinities despite the relatively large inter-individual variability (Figures 3 and 4; Table A4). The Mg/Ca ratios of the foraminiferal tests grown in the experiments show a slightly larger response to changes in salinity than previously observed by Nürnberg et al. (1996a) (values only from GAM specimens and chambers built during culture; in Table 1 data from Figures 3 and 6) (Figure 4A).

Table 3. Summary of Mg/Ca and Sr/Ca relationships with salinity, Ω and $[CO_3^{2-}]$[a].

	Regression	n	r^2	F	*p*	Experimentally determined responses (in mmol/mol) per salinity unit
Salinity vs. Mg/Ca (data from this study and Nürnberg et al., 1996)	Mg/Ca = 0.11*S + 1.00	31	0.30	10.43	0.003	0.11
	Regression	n	r^2	F	*p*	Experimentally determined responses(in mmol/mol) per 100 µmol/kg of $[CO_3^{2-}]$ and per Ω unit
$[CO_3^{2-}]$ vs. Sr/Ca	Sr/Ca = 0.001*$[CO_3^{2-}]$ + 1.15	29	0.40	18.30	<0.001	0.10
Ω vs. Sr/Ca	Sr/Ca = 0.04*Ω + 1.15	29	0.40	18.04	<0.001	0.04

[a]Temperature was kept constant at 26°C. All regressions and statistics are based on individual analyses (not means as plotted in the figures); "n" refers to the number of laser ablation analyses 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 Mg/Ca vs. salinity at constant Ω values and Sr/Ca vs. salinity at constant Ω values; *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 and Sr/Ca variability significantly well under the conditions analysed.

The changes in Mg/Ca ratios due to a salinity increase observed in the present study are smaller than the ones found in the planktonic foraminifera *Orbulina universa* and *Globigerinoides ruber* (white) (Lea et al., 1999; Kısakürek et al., 2008, respectively) (Figure 4A). The incorporation of Sr increases slightly with salinity (Figures 3D and E; 4B). In contrast, existing calibrations by Lea et al. (1999) and Kısakürek et al. (2008) showed that in cultured *O. universa* and *G. ruber* (white) Sr incorporation increases more rapidly with salinity changes (Figure 4B).

2.3.2 Effect of calcium carbonate saturation state (Ω) on Mg/Ca and Sr/Ca ratios

The incorporation of Mg into foraminiferal test carbonate increases at higher salinity values when Ω is kept constant (Figures 3A, B, and C). In contrast, no consistent relationship is observed between Sr/Ca ratios and salinity when Ω is kept constant at three different salinity values (Figures 3D, E, and F). When plotting foraminiferal Mg/Ca ratios versus Ω and

[CO_3^{2-}] no correlation is observed (Figures 5A and B, respectively). In comparison, there is a clear effect of Ω and [CO_3^{2-}] on foraminiferal Sr/Ca when salinity is kept constant at 36 (0.04 mmol/mol per Ω unit and 0.10 mmol/mol per 100 µmol/kg rise in [CO_3^{2-}]) (Table 3; Figures 5C and D).

2.4. Discussion

Several planktonic foraminiferal Mg/Ca – temperature calibrations have been published for different species during the last decade (Figure 2A) (Nürnberg et al., 1996b; Lea et al., 1999; Elderfield and Ganssen, 2000; Anand et al., 2003; Russell et al., 2004; McConnell and Thunell, 2005; Kısakürek et al., 2008). All these calibrations show an increase in the incorporation of Mg into foraminiferal test carbonate with increasing temperatures. Whereas most calibrations are based on traditional wet chemical analyses of complete dissolved foraminifera we used laser ablation ICP-MS for analyzing the trace elemental composition of single foraminiferal test chambers. We observe that Mg/Ca values measured on chambers 18 are higher whereas Mg/Ca values from chambers 19 are lower in comparison to existing *G. sacculifer* calibrations (Nürnberg et al., 1996b; Anand et al., 2003) (Figure 2B). Average Mg/Ca values, based on combining the data from chambers 18 and 19, is consistent with the existing whole-foraminifera calibrations (Figure 2B). This implies that differences in trace element composition between chambers can significantly affect trace element/Ca calibrations.

Such an impact of intra-chamber heterogeneity of foraminiferal Mg incorporation on reconstructions of past seawater temperatures was previously suggested by Eggins et al. (2003), Sadekov et al. (2005), Hintz et al. (2006) and Sadekov et al. (2008). Nevertheless, results from traditional Mg/Ca analyses (Anand et al., 2003) and laser ablation ICP-MS (this study) suggest similar foraminiferal Mg/Ca ratios for *G. sacculifer* at the same temperature (Figure 2B). Moreover, calibrations based on culturing experiments (this study and Nürnberg et al., 1996b) and those using core-top samples (Anand et al., 2003) are comparable (Figure 2B). This implies that laser ablation-ICP-MS as well as culture experiments are suitable approaches for investigating Mg incorporation in foraminiferal carbonate.
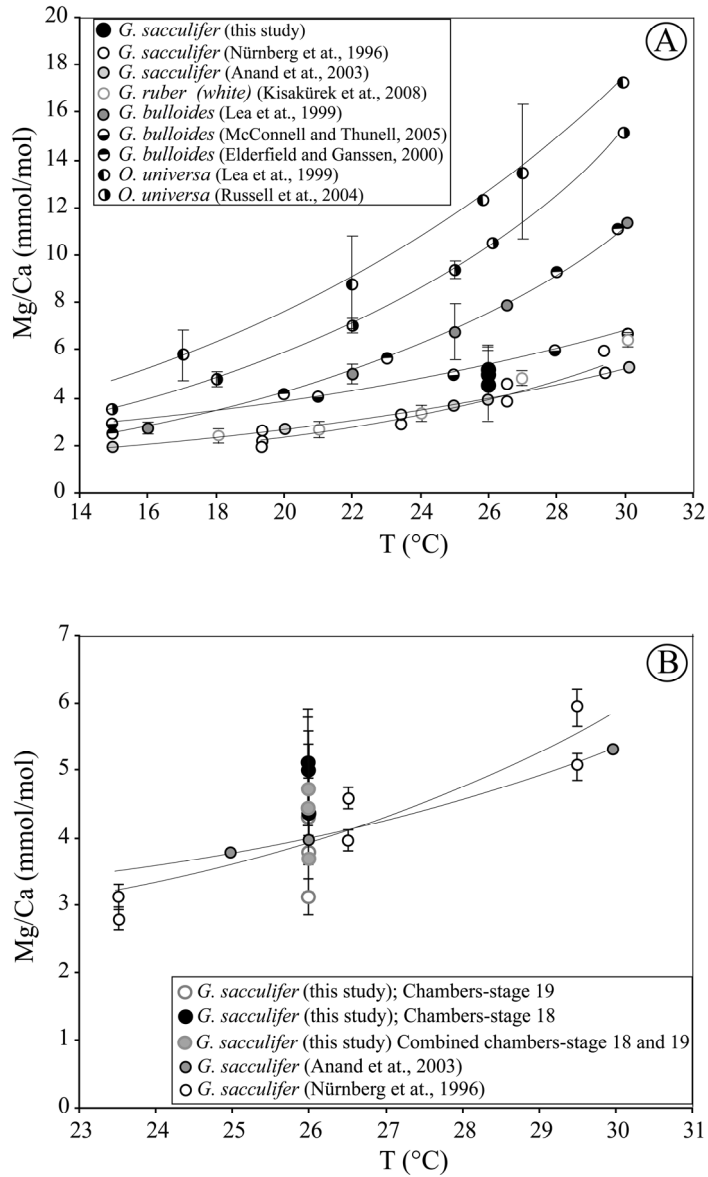
Figure 2. A. Mg/ Ca ratios of diverse planktonic foraminiferal shells from laboratory cultures and sediment traps vs. temperature. Salinity values vary from 30 to 39; B. Mg/Ca of *Globigerinoides sacculifer* from three different studies (data from present study correspond to chambers 18 and 19 from control experiments in which Ω was not modified; experiments 1, 5, and 9 from Table 2).

2.4.1 Effect of salinity on Mg/Ca and Sr/Ca ratios

The observed changes in planktonic foraminiferal Mg incorporation in response to different salinity values (Figure 3) can be due to a direct effect or to the related effect of salinity changes on the carbonate parameters. When we compare the results from the control experiments to those in which Ω was kept constant by modifying the carbonate chemistry over a salinity range (Table 2B; Figures 3A to 3C), we observe a similar positive correlation between Mg/Ca ratios and salinity. We also observe there is no significant effect of Ω within the range analysed (5.25-6.50) on foraminiferal Mg incorporation ($p > 0.05$) while a significant effect of salinity is found ($p < 0.05$). These results imply that changes in Mg/Ca ratios of *G. sacculifer* are mainly caused by salinity itself rather than Ω and the $[CO_3^{2-}]$ within the interval considered (salinity between 30 and 39). Therefore, the results discussed in the following section are based on the combined data of every salinity experiment (30, 36, and 39) regardless of their Ω value (Tables 2A and B; Figure 4A).

When plotting the data of Nürnberg et al. (1996a) together with our data, we observe that the Mg/Ca values of NO GAM specimens are below the general trend (points at salinities 26 and 35; Figure 4A). On the other hand, the Mg/Ca ratios at salinity "35" come from chambers that were grown in the natural environment which considerably varied in salinity (from 31.2 to 36) and temperature (from 26.2 to 29.7°C). This might explain the overall lower values at this salinity. Therefore, we have used the data of Nürnberg et al. (1996a) coming from GAM specimens and chambers grown in controlled culture experiments.

The increase in Mg/Ca ratios observed at higher salinities in our results (Figure 4A) is slightly larger than the one observed in a previous salinity calibration for *G. sacculifer* (Nürnberg et al., 1996a; from Table 1 data belonging to Figures 3 and 6). However, the 95% confidence interval from our data encompasses the Mg/Ca-salinity correlation of Nürnberg et al. (1996a). This indicates that the Mg incorporation observed in these two studies (present study and Nürnberg et al., 1996a) is at least statistically comparable (Figure 4A).

When we combine Mg/Ca ratios from the present study with the data from Nürnberg et al. (1996a), the resulting Mg/Ca-salinity relationship indicates that salinity only accounts for 30% of the variation in Mg/Ca ratios ($r^2 = 0.30$; Table 3).
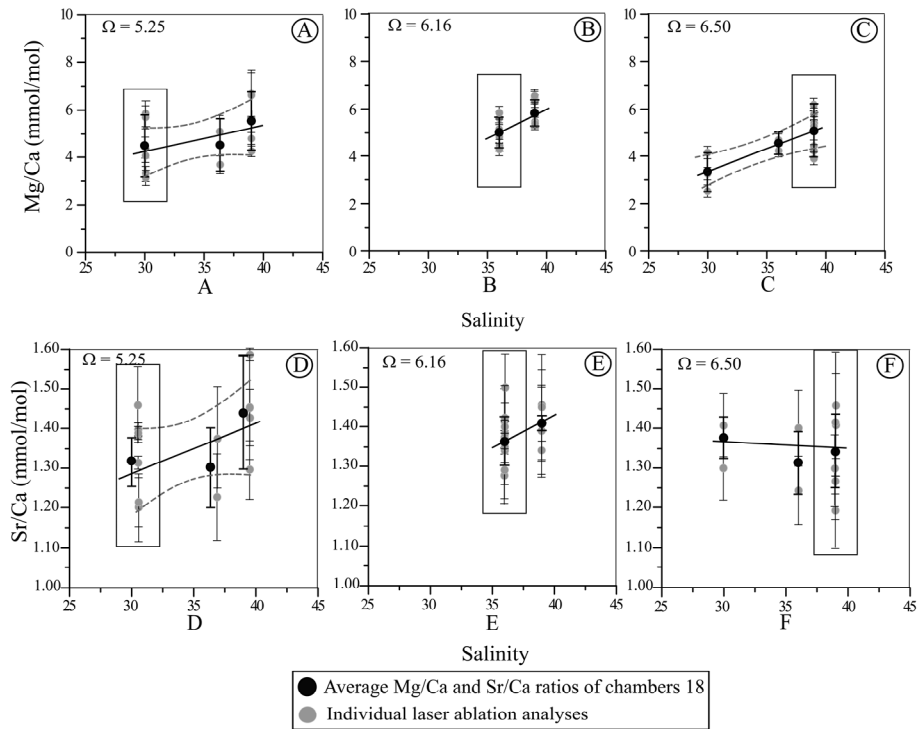
Figure 3. Foraminiferal Mg/Ca and Sr/Ca ratios at three different salinity values and a constant Ω. Mg/Ca: A. Ω = 5.25 ± 0.03 (from Tables 1 and 2 experiments No. 1, 2 and 3); B. Ω = 6.16 ± 0.02 (from Tables 1 and 2 experiments No. 4, 5 and 6) and, C. Ω = 6.50 ± 0.04 (from Tables 1 and 2 experiments No. 7, 8 and 9); Sr/Ca ratios from D to F, figures have the same correspondence as Mg/Ca ratios. Control experiments are marked with a square (from Tables 1 and 2 experiments No. 1, 5, and 9). The 95% confidence limits of the curve fit are shown by dashed lines.

This means that 70% of the variation in Mg/Ca ratios cannot be explained by salinity. Therefore, there must be other variables that have an influence too. We also observe that the regression model used provides a solid statistical basis to quantify the effect of salinity on Mg/Ca ($p < 0.05$; Table 3). From this Mg/Ca-salinity relationship, based on the combined data of this study and Nürnberg et al. (1996a), an increase of 0.11 mmol/mol of Mg/Ca per salinity unit is estimated (Table 3) which corresponds to an increase of 2.3% in Mg/Ca per salinity unit. This Mg/Ca response to salinity is slightly smaller than the ones found in the planktonic foraminifera *O. universa* and *G. ruber*, where Mg/Ca ratio dependencies on salinity of 4 and 5% respectively, are reported (Lea et al., 1999; Kısakürek et al., 2008; Figure 4A).

33
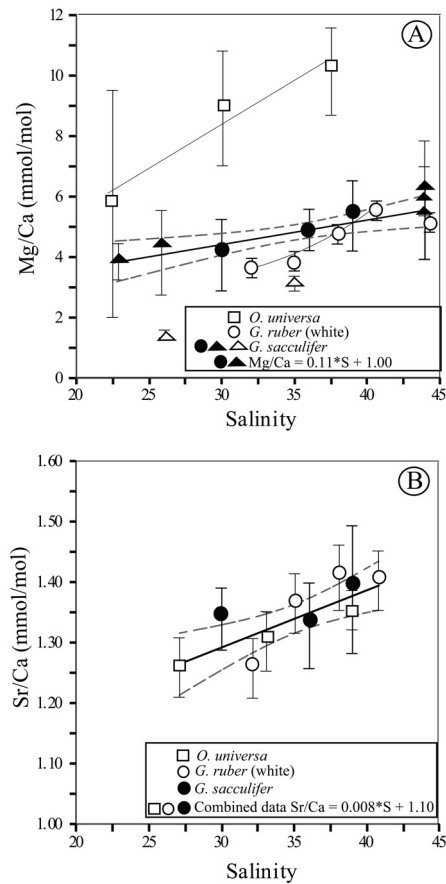
Figure 4. A. Foraminiferal Mg/Ca ratio vs. salinity; closed circles correspond to data from present study; open and closed triangles correspond to NO GAM and GAM specimens, respectively, obtained from Nürnberg et al. (1996a) and Nürnberg's pers. comm; open circles correspond to data from Kısakürek et al. (2008); open squares correspond to data from Lea et al. (1999); B. Foraminiferal Sr/Ca ratio vs. salinity; symbols have the same correspondence as in Figure 4A. Data from present study correspond to Mg/Ca and Sr/Ca averages of every salinity experiment regardless of the Ω value (Tables 2A and B). Error bars are standard deviations based on the spread and number of analyses per experiment (applied to all figures). The 95% confidence limits of the curve fit are shown by dashed lines.

The most important factor influencing Mg/Ca ratio of inorganic calcite is the Mg/Ca ratio of the parent solution from which calcite precipitates (Mucci and Morse, 1983). The much depleted Mg/Ca ratios of foraminiferal calcite, therefore, suggest a strongly modified internal calcification pool. Although increasing salinity of the seawater does not change the Mg/Ca ratio of the parent solution, absolute concentrations of

these two elements do change. According to Bentov and Erez (2006), there is an inward driving force for Mg^{2+} from the extracellular environment into the cell. This has only been established in eukaryotic cells but it is probable that this is not significantly different in foraminifera. In order to keep a low intracellular Mg^{2+} activity, as other eukaryotes do, foraminifera may use different cellular mechanisms such as Mg protein-based transport systems, cellular buffering and/or sequestration within cellular organelles (Bentov and Erez, 2006).

At higher salinities the parent solution from which calcite precipitates has a higher Mg^{2+} concentration. This parent solution is kept in an extracellular space from which the cell will extract Mg^{2+} to the cytoplasm by using Mg channels and pumps (Erez, 2003). We assume that the amount of Mg^{2+} extracted from this parent solution does not vary and the foraminifer continues to use the same amount of energy in order to lower the Mg^{2+} of the parent solution. If this is the case, higher Mg^{2+} in the parent solution would be reflected in the shell composition (Segev and Erez, 2006), which is in line with the observations from the present study (Figures 3A, B, and C; 4A). Higher amounts of extracted Mg^{2+} coming from the parent solution into the foraminifer cytoplasm would mean an increase in the energy required to extrude this excess of Mg^{2+} from the cell (Zeebe and Sanyal, 2002). This strategy would not be as cost-effective as extracting the same $[Mg^{2+}]$ regardless of the element concentration of the parent solution. This assumption is in line with inorganic calcite precipitation studies by Zeebe and Sanyal (2002) in which it is shown that, in order to initiate the calcification process at biogenic precipitation rates, the best cost-effective strategy is H^+ removal from the calcifying area. Therefore, the foraminifer would not change the rate at which Mg^{2+} is extruded from the cell in order to calcify.

The mechanisms responsible for the influence of salinity on Mg/Ca ratios are not well understood. Therefore, the difference found in the Mg/Ca ratio response to salinity changes between planktonic foraminiferal species cannot be explained with confidence (Figure 4A). We suggest it might be due to the specific effect that a salinity increase has on the cellular Mg channels and pumps. In the case of *O. universa* and *G. ruber* (white), the biological pumps in charge of extracting Mg^{2+} from the parent solution could be less tolerant and therefore, less efficient at higher salinities than the Mg pumps of *G. sacculifer*. This would cause higher than usual Mg^{2+} in the parent solution that would be reflected in the biogenic calcite. We also suggest that the different Mg/Ca-salinity correlations found in planktonic foraminifera might be caused by the difference in salinity dependence between Mg/Ca ratios of GAM calcite and
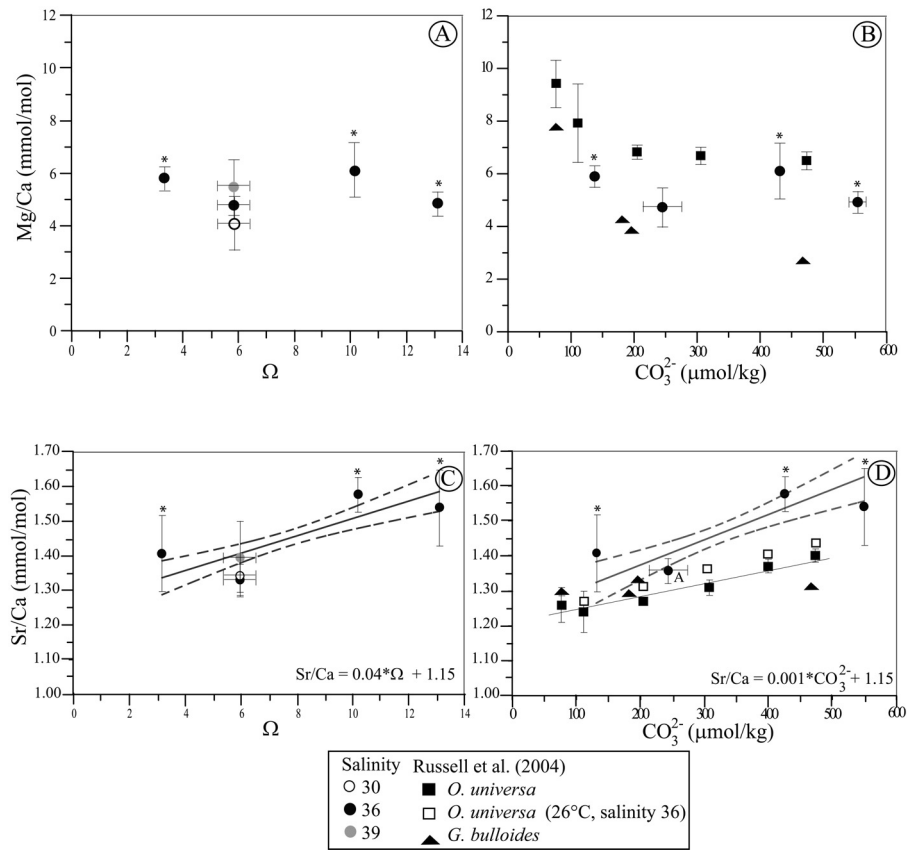
Figure 5. A and B. Foraminiferal Mg/Ca ratio at different Ω and [CO_3^{2-}] values respectively (constant temperature of 26 °C); C and D. Foraminiferal Sr/Ca ratio at different Ω and [CO_3^{2-}] values (constant temperature of 26 °C). The 95% confidence limit of the curve fit is shown by dashed lines. [A]Average of Sr/Ca of salinities 30, 36, and 39;* data from da Rocha et al. in prep (Table A6).

Mg/Ca values of NO GAM calcite. The Mg/Ca ratios from chamber stage 18 (F-1 chambers) have a significant correlation with salinity (Table 3), while Mg/Ca ratios from chamber stage 19 (sac-like/F chambers -most of it GAM calcite-) do not show significant correlation with this parameter (data not included).

This indicates that the response of Mg/Ca ratios from chambers 18 to salinity might be slightly biased by the presence of GAM calcite. As a result, the differences in Mg/Ca response to salinity between planktonic species with and without GAM calcite become larger (e.g. Mg/Ca salinity

dependencies of 2.3% and 5% per salinity unit for *G. sacculifer* and *G.ruber*, respectively).

There is no consistent relationship between Sr/Ca ratios and salinity within the salinity range analysed, 30 to 39 (Table 2B, Figures 3D, E, and F; 4A). We also observe there is no significant effect of Ω within the range analysed (5.25-6.50) on foraminiferal Sr incorporation ($p >$ 0.05). Therefore, the results discussed in the following section are based on the combined data of every salinity experiment, regardless of their Ω values (Tables 2A and B; Figure 4B). A slight increase in Sr incorporation in the test carbonate of *G. sacculifer* is observed at higher salinity values (Figure 4B). Lea et al. (1999) and Kısakürek et al. (2008) also reported a positive correlation between Sr/Ca and salinity for the planktonic foraminifera *O. universa* and *G. ruber* (white), respectively.

The 95% confidence interval from our data encompasses the previous Sr/Ca-salinity correlations of the two other species of planktonic foraminifera which means that Sr incorporation in these three planktonic species is, at least statistically, comparable and thus potentially reflects similar processes related to salinity increases. When combining Sr/Ca ratios from the present study with the data from Lea et al. (1999) and Kısakürek et al. (2008) (Figure 4B), an increase of 0.008 mmol/mol of Sr/Ca per salinity unit is observed. According to Lorens (1981), Nehrke et al. (2007) and Tang et al. (2008), higher calcite Sr/Ca ratios are mainly associated with higher growth rates in inorganic calcite which is also observed in biogenic calcite (Kısakürek et al., 2008). Results from inorganic experiments (Zuddas and Mucci, 1998) furthermore indicate a positive linear correlation between calcite precipitation rate and ionic strength of the solution. Therefore, the observed increase in Sr/Ca values at higher salinities (Figure 4B) might primarily result from an increased in Ω.

2.4.2 Effect of calcium carbonate saturation state (Ω) on Mg/Ca and Sr/Ca ratios

There is no clear effect of either Ω or $[CO_3^{2-}]$ on foraminiferal Mg incorporation when salinity is kept constant at 36 (Figures 5A and B). This might be due to the selected range of Ω and $[CO_3^{2-}]$ values, which was rather limited (Table 2A). Russell et al. (2004) showed that the Mg/Ca-$[CO_3^{2-}]$ relationship in planktonic foraminifera tends to have a relatively constant value above CO_3^{2-} concentrations of 200 µmol/kg (Figure 5B), while below this concentration it rapidly increases. Most of the Mg/Ca ratio

data for *G. sacculifer* fall in the interval where no significant variations are expected (Figure 5B).

Omega has a stronger effect on Sr incorporation in *G. sacculifer* than salinity with an increase of 0.04 mmol/mol of Sr/Ca per Ω unit for a constant salinity of 36 compared to 0.008 mmol/mol of Sr/Ca per salinity unit (Table 3; Figures 4B and 5C). This Sr/Ca- Ω relationship indicates that Ω accounts for almost half of the variation in Sr/Ca ratios ($r^2 = 0.40$; Table 3). We also observe that the regression model predicts a statistically significant Sr/Ca variability due to changes in Ω ($p < 0.001$; Table 3). In inorganic and biogenic calcite, the Sr/Ca ratio is known to increase with growth rate (Lorens, 1981; Nehrke et al., 2007; Tang et al., 2008; Kısakürek et al., 2008), and a higher $[CO_3^{2-}]$ increases foraminiferal shell weight and possibly calcification rates (Bijma et al., 1999). Therefore, the observed correlation is possibly linked to the calcification rate.

We observe a positive correlation between Sr/Ca ratios and $[CO_3^{2-}]$ (or pH) in the planktonic species *G. sacculifer* (this study) and *O. universa* (Russell et al., 2004) (Figure 5D). However, the Sr/Ca sensitivities found in these two planktonic foraminifera are different (0.10 mmol/mol of Sr/Ca per 100 µmol/kg rise in $[CO_3^{2-}]$ for *G. sacculifer*, and 0.04 mmol/mol of Sr/Ca per 100 µmol/kg rise in $[CO_3^{2-}]$ for *O. universa*). *G. sacculifer* Sr/Ca values are also overall higher than those of *O. universa* (Figure 5D). This interspecific variability in Sr/Ca is too large to be caused by differences in salinity and temperature between culturing experiments alone (Figure 5D; cf. Russell et al. (2004) for temperature effect and Lea et al. (1999) for salinity effect on Sr/Ca in *O. universa*). Therefore, the response of Sr/Ca to increasing $[CO_3^{2-}]$ appears to be species-specific. According to Anderson and Faber (1984), Lea et al. (1995) and Bijma et al. (1999), *G. sacculifer* has a higher calcification rate than *O. universa*. The rates at which these two species calcify might thus be responsible for both the difference in Sr/Ca ratios and the different response of foraminiferal Sr incorporation to $[CO_3^{2-}]$ changes (Figure 5D). The presence and size of internal DIC and calcium pools in the cytoplasm of *G. sacculifer* might also affect Sr incorporation into the test calcite and could also be responsible for the interspecies variability in Sr/Ca ratios. However, precipitation of trace elements from these internal reservoirs and their chemical composition are difficult to study (Erez, 2003).

Differences in the response of Sr/Ca to $[CO_3^{2-}]$ (or pH) between *G. sacculifer* and *G. bulloides* (Figure 5D) might be related to the impact of photosynthetic symbionts in *G. sacculifer* on the calcification rate. According to the foraminiferal biomineralization model proposed by Wolf-Gladrow et al. (1999a), photosynthetic uptake of CO_2 in the foraminifer

microenvironment leads to an increase in pH and $[CO_3^{2-}]$, which potentially contributes to overall higher calcification rates (Bijma et al., 1999; Lea et al., 1999).

2.4.3 Paleoceanographic implications

Combining present and published data, we infer that a salinity increase of 4 units is equivalent to a 1°C temperature increase, using the Mg/Ca ratio temperature calibration from Nürnberg et al. (1996b): Mg/Ca = 0.3948 exp (0.0888*T). Although smaller than the effect of sea surface temperature (SST), salinity has a limited though significant impact on planktonic foraminiferal Mg incorporation, sometimes with important consequences for the interpretation of paleorecords (e.g. Groeneveld et al., 2008). According to Groeneveld et al. (2008), salinity variations of up to three units, based on the planktonic $\delta^{18}O$ record during the latest Miocene/Early Pliocene (5.6-3.9 Ma), would result in an overestimation of foraminiferal Mg/Ca ratios of ~21%. This offset is based on the original Mg/Ca-salinity calibration of Nürnberg et al. (1996) using both GAM and NO GAM specimens (Table 1 data belonging to Figure 6). Our salinity calibration, based on culture experiments of GAM specimens of *G. sacculifer*, suggests a more modest overestimation of Mg/Ca ratios of ~7%. This implies that, although the overall conclusion remains the same, the Caribbean temperature increase after 4.5 Ma was probably somewhat larger.

Although the impact of salinity on Mg/Ca ratios is limited in today's ocean, glacial-interglacial changes in salinity potentially offset temperature reconstructions. Whereas the effect of ice volume alone is about 1.2 salinity units, the drier tropical climate regionally impacted salinity as well. In the tropical western Atlantic Ocean, for instance, sea surface salinity changed between 1 and 3 units approximately from the Last Glacial Maximum (LGM) to the Holocene (Toledo et al., 2007; Weldeab et al., 2006). When comparing these salinity changes with reconstructed equatorial Atlantic SSTs during the LGM (3°C cooler than SSTs during the Holocene) (Toledo et al., 2007; Weldeab et al., 2006), we find that 25% of this temperature decrease might actually be caused by salinity. According to our results, an increase of 4 salinity units is equivalent to about 1°C warming, in terms of Mg/Ca ratios. Consequently, a change of 3 salinity units, during the LGM-Holocene period, would cause an offset of 0.75°C, which equals 25% of the total $\Delta SST_{Mg/Ca}$ observed between Holocene and LGM sea surface temperatures. We consider this a

significant offset that should be taken into account when calculating the $SST_{Mg/Ca}$ of the tropical Atlantic Ocean during the LGM.

The positive correlation between Ω ($[CO_3^{2-}]$ and thus pH) and Sr/Ca of symbiont-bearing planktonic species such as *G. sacculifer* (present study) and *O. universa* (Russell et al., 2004) could potentially bias data of past changes in seawater Sr/Ca (Stoll et al., 1999). The observed changes in foraminiferal Sr/Ca ratios coinciding with glacial-interglacial cycles are partly attributed to past changes in seawater Sr/Ca ratios and partly to changes in pCO$_2$. Our results as well as the culture study by Russell et al. (2004) demonstrate that changes in Ω ($[CO_3^{2-}]$ and hence pH) have an important impact on foraminiferal Sr incorporation: almost half of the Sr/Ca variability observed in the present study can be explained by changes in Ω ($[CO_3^{2-}]$ and thus pH) (Figures 5C and D; Table 3). Reconstructions of glacial-interglacial changes in seawater $[CO_3^{2-}]$, based on δ^{13}C records of *G. sacculifer* and *G. ruber* (Spero et al., 1999), indicate a broad variation in $[CO_3^{2-}]$ of about 50 μmol/kg during the Last Glacial Maximum (LGM). According to the present study, this change in seawater $[CO_3^{2-}]$ would correspond to a 0.05 mmol/mol change in foraminiferal Sr/Ca ratios from *G. sacculifer*. Our correlation between Sr/Ca and seawater $[CO_3^{2-}]$ could potentially fully explain the observed glacial to interglacial changes in Sr/Ca ratios of *G. sacculifer* (Stoll et al., 1999), without changing seawater Sr/Ca ratios. Therefore, when studying foraminiferal Sr/Ca records on longer timescales, changes in ocean carbonate chemistry must be considered in addition to variations in seawater Sr/Ca.

2.5. Conclusions

The present study corroborates the presence of a salinity effect on Mg incorporation in *G. sacculifer*, although it is considerably smaller than the temperature effect (only 30% of the variation in Mg/Ca ratios in our experiments is due to salinity). The Mg/Ca ratios of *G. sacculifer*, this study and Nürnberg et al. (1996a), show a 0.11 mmol/mol increase per salinity unit, which implies a salinity increase of 4 units corresponding to a 1°C temperature increase. This effect of salinity on Mg incorporation is relatively modest. However, when using Mg/Ca-based temperatures in combination with foraminiferal δ^{18}O to calculate salinity, it can not be neglected. Due to changes in salinity, on a glacial-interglacial timescale tropical $\Delta SST_{Mg/Ca}$ can be offset by up to 1°C which corresponds to a 25% bias in the total $\Delta SST_{Mg/Ca}$. At constant temperature of 26°C, our results suggest that salinity controls the Mg incorporation, as no impact of

carbonate chemistry was observed within the range of Ω and $[CO_3^{2-}]$ analysed (Ω = 5.25 to 6.50; $[CO_3^{2-}]$ = 218 to 270 µmol/kg). Seawater Ω ($[CO_3^{2-}]$ and thus pH) appears to be the main control on shell Sr incorporation (0.10 mmol/mol per 100µmol/kg rise in $[CO_3^{2-}]$), resulting in an indirect salinity dependence of Sr/Ca of 0.008 mmol/mol per salinity unit.

Calcite precipitation and growth rates of planktonic foraminifera *G. sacculifer* might be influenced by changes in environmental conditions, processes that may be reflected in the Sr composition of their carbonate test. Variations in the environment of calcification such as changes in carbonate chemistry of the surrounding seawater can also potentially offset past reconstructions of glacial-interglacial seawater Sr/Ca ratios. This might be caused by the influence of Ω and the $[CO_3^{2-}]$ on foraminiferal Sr incorporation (0.04mmol/mol per Ω unit and 0.10 mmol/mol per 100 µmol/kg rise in $[CO_3^{2-}]$; for the planktonic species *G. sacculifer*).

Acknowledgements

Chapter 3

The impact of seawater calcite saturation state by modifying Ca ion concentrations on Mg and Sr incorporation in cultured benthic foraminifera

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Abstract

We investigated the effect of the calcium concentration in seawater and thereby the calcite saturation state (Ω) on the magnesium and strontium incorporation into benthic foraminiferal calcite under laboratory conditions. For this purpose individuals of the shallow-water species *Heterostegina depressa* (precipitating high-Mg calcite, symbiont-bearing) and *Ammonia tepida* (low-Mg calcite, symbiont-barren) were cultured in media under a range of $[Ca^{2+}]$, but similar Mg/Ca ratios. Trace element/Ca ratios of newly formed calcite were analysed with Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) and normalized to the seawater elemental composition using the equation D_{TE} = $(TE/Ca_{calcite})/(TE/Ca_{seawater})$. The culturing study shows that D_{Mg} of *A. tepida* significantly decreases with increasing Ω at a gradient of $-4.3*10^{-5}$ per Ω unit. The D_{Sr} value of *A. tepida* does not change with Ω, suggesting that fossil Sr/Ca in this species may be a potential tool to reconstruct past variations in seawater Sr/Ca. Conversely, D_{Mg} of *H. depressa* shows only a minor decrease with increasing Ω, while D_{Sr} increases considerably with Ω at a gradient of 0.009 per Ω unit. The different responses to seawater chemistry of the two species may be explained by a difference in the calcification pathway that is, at the same time, responsible for the variation in the total Mg incorporation between the two species. Since the Mg/Ca ratio in *H. depressa* is 50-100 times higher than that of *A. tepida*, it is suggested that the latter exhibits a mechanism that decreases the Mg/Ca ratio of the calcification fluid, while the high-Mg calcite forming species may not have this physiological tool. The dependency of Mg incorporation on seawater $[Ca^{2+}]$ in low-Mg species shows the need to correct for the calcium concentration in order to reconstruct reliable temperatures from fossil Mg/Ca ratios.

3.1. Introduction

The ratio of magnesium to calcium (Mg/Ca) in fossil benthic foraminiferal shells is widely used to estimate past bottom water temperatures (Lear et al., 2000; Martin et al., 2002; Billups and Schrag, 2003; Katz et al., 2008; Kristjánsdóttir et al., 2007; Lear et al., 2003b; Shevenell et al., 2008; Skinner et al., 2003). The applicability of Sr/Ca ratios in foraminiferal calcite is less well defined. Whereas Sr/Ca variations in benthic foraminifera have been attributed to changes in Sr/Ca of seawater (Lear et al., 2003a), hydrostatic pressure (Elderfield et al., 1996), and temperature and/or carbonate ion concentration (Reichart et al., 2003; Rosenthal et al., 2006), those in planktonic species were found to depend on pH and/or [CO$_3^{2-}$] (Dueñas-Bohȯrquez et al., 2009; Lea et al., 1999; Russell et al., 2004), and growth rate (Kısakürek et al., 2008).

The Mg/Ca ratio of foraminiferal test carbonate has been used in combination with δ^{18}O to reconstruct past δ^{18}O of seawater (e.g., Lear et al., 2000; Billups and Schrag, 2003; Shevenell et al., 2008). However, recent studies have shown that in addition to temperature the calcite saturation state (Ω or Δ[CO$_3^{2-}$]) potentially plays an important role in the Mg uptake into benthic foraminiferal shells (Elderfield et al., 2006; Healey et al., 2008; Rosenthal et al., 2006; Raitzsch et al., 2008). Calibrations using foraminifers from surface sediments yield a species-specific increase in Mg/Ca between 0.008 and 0.017 mmol/mol per µmol/kg Δ[CO$_3^{2-}$]. Given the empirical exponential relationship, foraminiferal Mg/Ca changes only slightly within the generally narrow temperature range in the deep ocean of roughly 5°C. On the other hand, potential changes in deep-sea Δ[CO$_3^{2-}$], for example in the North Atlantic during the Last Glacial Maximum by ~25-30 µmol/kg, inferred from benthic foraminiferal B/Ca (Yu and Elderfield, 2007), would considerably bias Mg/Ca-based temperature estimations. Hence, for the ongoing development of this paleo-thermometer, it is essential to quantify the separate effects of temperature and calcite saturation state on benthic Mg/Ca ratios. Interestingly, field studies on benthic foraminifera contradict results from culturing experiments on planktonic foraminifera, which show that Mg/Ca decreases with increasing pH or [CO$_3^{2-}$] (i.e. an increase in calcite saturation state; Kısakürek et al., 2008; Lea et al., 1999; Russell et al., 2004).

The saturation state of seawater with respect to calcite is defined as Ω=[Ca^{2+}]*[CO$_3^{2-}$]/K$_{sp}$, where K$_{sp}$ corresponds to the solubility product of calcite, that depends on ambient temperature, salinity, and pressure (Zeebe and Wolf-Gladrow, 2001). Accordingly, Ω changes when [CO$_3^{2-}$]

and/or [Ca^{2+}] change, but in the open ocean the calcium concentration is relatively constant with a long residence time of approximately 1.1 Ma (Broecker and Peng, 1982). Therefore, the calcite saturation state in seawater mainly varies with water depth and the carbonate ion concentration [CO_3^{2-}], which is intrinsically linked to the other carbonate system parameters (i.e. DIC, alkalinity, and pH; Zeebe and Wolf-Gladrow, 2001). However, a number of culturing studies have been conducted to determine the carbonate ion effect on foraminiferal Mg/Ca and Sr/Ca (Kısakürek et al., 2008; Lea et al., 1999; Russell et al., 2004; Dissard et al., 2009a; Dueñas-Bohórquez et al., 2009), but only one on the effect of [Ca^{2+}] (Bentov and Erez, 2006). Hence, it is crucial to understand whether varying [Ca^{2+}] yields similar changes in the Mg and Sr incorporation as varying [CO_3^{2-}], and whether the fractionations are constant between taxa.

For these reasons, we analysed Mg/Ca and Sr/Ca ratios in two species of foraminifera with contrasting calcification mechanisms, in relation to the calcium ion effect by means of controlled culturing experiments. For this study, we used the neritic to intertidal benthic species *Heterostegina depressa* (high-Mg calcite, tropical, symbiont-bearing) and *Ammonia tepida* (low-Mg calcite, temperate, symbiont-barren). Compared to deep-sea foraminifera, intertidal species are resistant to environmental fluctuations and can therefore grow at a range of experimental conditions. In our experiments, we varied the $CaCO_3$ saturation state by altering the calcium concentration [Ca^{2+}] of the medium. Under natural conditions, the [Ca^{2+}] covaries with salinity. By keeping salinity constant in the cultures, while varying [Ca^{2+}], it is also possible to deconvolve the effect of Ω from salinity on e.g. Mg incorporation.

3.2. Methodology

3.2.1. Sample collection and preparation

Specimens of the shallow benthic species *A. tepida* were isolated from sediment collected at the Dutch Wadden Sea and stored in the laboratory at 18°C. Specimens of the symbiont-bearing species *H. depressa*, provided by Burger's Zoo (Arnhem, The Netherlands), were picked from stocks kept at 24°C and under a 12 hour light:12 hour dark cycle (De Nooijer et al., 2007).

Living individuals bearing brightly coloured cytoplasm were selected and placed in vials with natural seawater admixed with calcein

(fluorexon, fluorescein complex) at a final concentration of 7 mg/L. The fluorescent indicator calcein is incorporated into the calcite walls whenever new chambers are formed, whereas pre-existing chambers are not affected (Bernhard et al., 2004). Incubated specimens can therefore be scanned afterwards to determine which chambers were built during the culturing period (Figure 1).
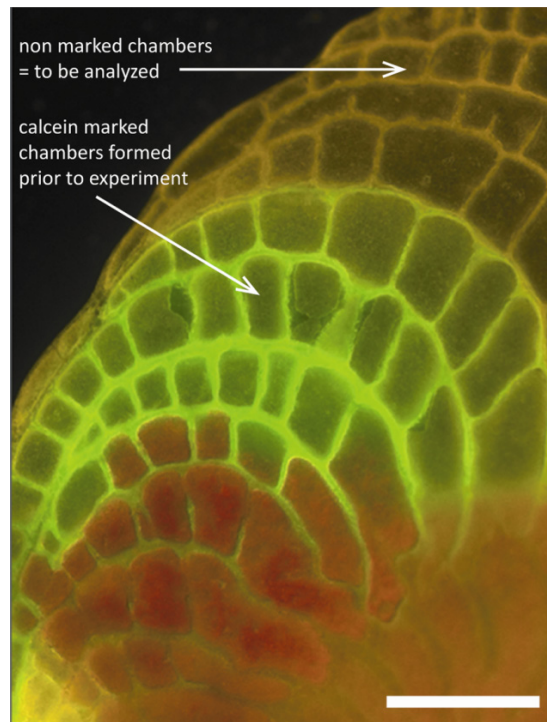
Figure 1. *Heterostegina depressa* under a fluorescence microscope after excitation. Shell calcite marked with calcein (green) was built prior to the experiment. The younger (newly formed) non-marked chambers were formed during the experiment and were analysed with LA-ICP-MS. Scale bar is 100 µm.

After being maintained in an incubator for 2-4 weeks, foraminifers were checked for new chambers under an inverted fluorescence microscope. Only specimens that had chambers clearly marked by calcein were selected for the culturing experiments. The chambers formed during the experiment could be easily identified (Figure 1). Although it was recently established that calcein does not affect the incorporation of trace metals (Dissard et al., 2009b), not admixing calcein to the culture water during the experiments avoided any possible impact.

Upon finishing the experiments, only foraminifers with new, non calcein-labelled chambers were selected for elemental analysis with laser ablation-ICP-MS. The specimens were placed in a sodium hypochlorite bath (NaOCl 5%) for 10 minutes to remove cytoplasm and organic material from the surface of the tests. The shells were then thoroughly washed with deionised water and dried.
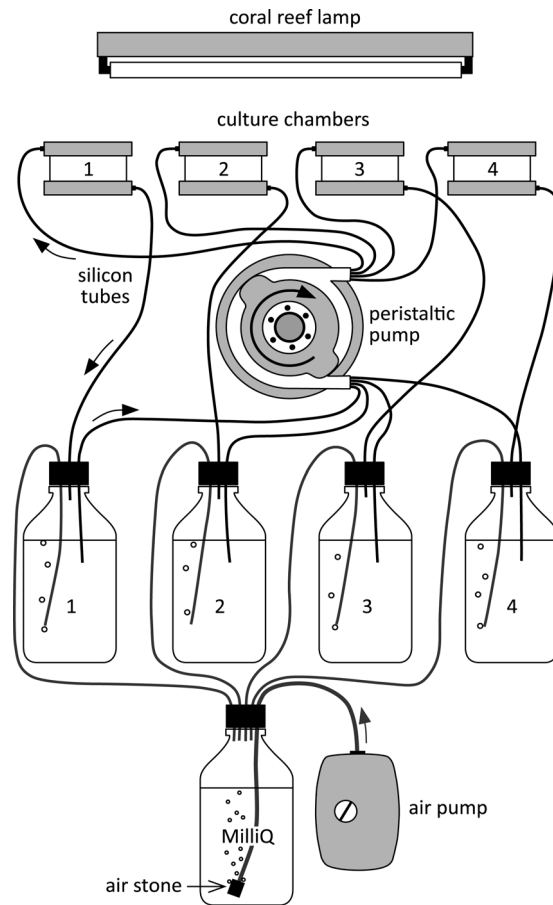
Figure 2. Schematic illustration of the instrumental setup. See main text for details.

3.2.2. Experimental setup

Four seawater solutions were prepared with different Ω values. We varied parameters based on the following relationship:

$$\Omega = \frac{[CO_3^{2-}]*[Ca^{2+}]}{K_{sp\,(Cc)}^{*}} \qquad \text{(Equation 1)}$$

where Ω is the calcite saturation state of the seawater and $K^*_{sp(cc)}$ is the solubility product of calcite.

Since we altered the chemical composition of seawater for our experiments, the solutions were composed of 50% natural seawater (NSW) from the eastern Mediterranean Sea and 50% artificial seawater (ASW). The ASW was prepared according to the methods of Kester et al. (1967) and Berges et al. (2001). Addition of the volumetric salts $CaCl_2$ (1M) and $MgCl_2$ (1M) ensured similar Mg/Ca ratios (approximately 5.2) between the different solutions (Table 1). Since Cl$^-$ is the major anion in seawater, different amounts of $CaCl_2$ and $MgCl_2$ were compensated by accordant addition of NaCl (2M). In this manner, salinity was kept constant between the media (Table 1).

All seawater solutions were filtered using a 0.2 µm filter and were air-bubbled for 24 hrs in order to equilibrate the seawater with atmospheric pCO$_2$. Finally, four individual solutions were obtained with the following approximate calcium concentrations: 1/2 of natural [Ca^{2+}] (group 1), natural [Ca^{2+}] (group 2), 1.5x natural [Ca^{2+}] (group 3), 2x natural [Ca^{2+}] (group 4); see Table 1 for exact concentrations.

Specimens of *H. depressa* and *A. tepida* (15 and 25-30 individuals, respectively) were incubated in 30 ml containers termed culture chambers throughout this paper. These containers had a polyacetal bottom and lid, separated by a diaphanous Plexiglas ring (Figure 2). At the bottom of the culture chamber a 0.2 µm filter (cellulose acetate) was placed, on top of which a thin layer of artificial sediment (SiO$_2$) was added. A small amount of a freeze-dried mixture of microalgae/diatom (*Dunaliella salina* and *Phaeodactylum tricornutum*) was added as food. A constant flow of seawater refreshed the culture chambers during the duration of the experiment (Figure 2). The chambers were connected with silicon tubes to reservoirs of 1 l glass bottles that contained the seawater with modified carbonate chemistry. A peristaltic pump circulated the modified seawater through the system at a speed of approximately 1 ml/h while the media was bubbled continuously with moist air (Figure 2). Shortly before setting up the experiments, all material was thoroughly cleaned several times with HCl (1N) and deionised water, and subsequently rinsed with the seawater used for that experimental treatment.

The complete system was placed in an incubator and kept at constant temperature (±1°C). The experiment containing specimens of *A. tepida* was kept at 18°C and in the dark, while the *H. depressa* experiment was conducted at 24°C and exposed to an artificial light cycle of 12 hrs light/12 hrs dark to allow for photosynthesis of their endosymbionts. Each experiment ran for two months. At the beginning

and at the end of each experiment, seawater was subsampled for determining elemental concentrations with ICP-OES, and for alkalinity analyses using an automated titrator (702 SM Titrino, Metrohm).

Table 1. Experimental culturing conditions.

Experiment I *H. depressa*	Group 1	Group 2	Group 3	Group 4
Temperature (°C)	24	24	24	24
Salinity	36.2	35.8	35.6	35.6
Alkalinity (µmol/kg)	2440±123	2436±112	2400±50	2384±80
DIC (µmol/kg)[a]	2087±102	2085±74	2059±34	2046±47
$[Ca^{2+}]$ (µmol/kg)	4847±28	9534±374	13760±233	18439±242
$[CO_3^{2-}]$ (µmol/kg)[a]	246±25	243±22	237±10	234±15
Ω[b]	2.76±0.11	5.39±0.51	7.58±0.29	10.04±0.56
Mg/Ca_{sw}	5.17±0.03	5.56±0.09	5.96±0.14	6.20±0.33
$Sr/Ca_{sw}*1000$	17.82±0.42	9.34±0.27	6.43±0.05	4.81±0.14
Experiment II *A. tepida*	Group 1	Group 2	Group 3	Group 4
Temperature (°C)	18	18	18	18
Salinity (psu)	35.2	35.3	35.2	35.3
Alkalinity (µmol/kg)	2385±29	2378±23	2363±31	2385±39
DIC (µmol/kg)[a]	2111±2	2105±16	2093±13	2111±11
$[Ca^{2+}]$ (µmol/kg)	5427±17	9786±5	14146±428	18138±539
$[CO_3^{2-}]$ (µmol/kg)[a]	201±4	200±3	198±4	200±6
Ω[b]	2.51±0.10	4.51±0.10	6.46±0.38	8.37±0.47
Mg/Ca_{sw}	5.08±0.02	5.10±0.10	5.19±0.21	5.30±0.28
$Sr/Ca_{sw}*1000$	15.45±0.62	8.46±0.10	5.87±0.01	4.58±0.11

Values are given with ± standard deviations.
[a]DIC and $[CO_3^{2-}]$ were calculated from pCO_2 and alkalinity using the *CO_2sys* program (Pierrot et al., 2006), using constants from Mehrbach et al. (1973) and pressure corrections of the equilibrium constants from Millero (1983).
[b]Ω is the product of $[CO_3^{2-}]$ and $[Ca^{2+}]$, divided by the solubility product of calcite (see Equation 1).

Salinity was checked monthly and adjusted when required. As seawater was in equilibrium with the atmosphere, a pCO_2 value of 365 µatm (Striegl et al., 2001) was used to calculate the other carbonate system parameters using the *CO_2sys* software (Pierrot et al., 2006).

3.2.3. Element analysis

Element concentrations in the test carbonate of single chambers of the cultured specimens were determined using a GeoLas 200Q 193nm Excimer laser (Lambda Physik) connected to a quadrupole ICP-MS

(Reichart et al., 2003). Only newly formed chambers of the foraminifers were ablated, identifiable from non calcein-marked chambers on photographs taken with a fluorescence microscope. Beam diameter was set at 60 µm for *A. tepida* and 80 µm for *H. depressa*, repetition rate was 6 Hz, and energy density was set at 1 J/cm^2. Element concentrations were calculated from isotopic counts for ^{24}Mg, ^{26}Mg, ^{27}Al, ^{42}Ca, ^{43}Ca, ^{44}Ca, ^{55}Mn, and ^{88}Sr, where Al and Mn were monitored as indicators for contaminant phases. Although the foraminifers were cultured without natural sediment, some contamination from an unknown source was observed and parts of the obtained ablation profiles displaying contaminants were discarded before calculating element/Ca ratios.

Calcium was used as an internal standard assuming 40%wt, which enables to correctly calculate element to calcium ratios routinely reported in paleoceanographic studies. Before and after ~10 sample analyses, a NIST 610 silicate standard with precisely determined elemental concentrations (Pearce et al., 1997) was measured three times each as an external standard. Since the NIST was ablated with a higher energy density (4 J/cm^2) in order to enhance signal quality, an in-house Iceland spar calcite ablated with low energy was employed as second, matrix-matched standard. However, calibration of many elements such as Mg, Ca, B, U, and Sr in carbonates against the NIST 610 were proven accurate when using a 193nm laser, even though instrumental settings were changed between the glass standard and carbonate samples (Hathorne et al., 2008).

Time resolved raw data in counts per second (cps) were converted to element concentrations (ppm) using the GLITTER software (New Wave Research, Inc.). This data reduction software also facilitates the manual selection of intervals used for background subtraction and signal integration.

3.3. Results

3.3.1 Survival and growth rates

The survival rate for both species was very high at almost 100% in all experiments. Three specimens (out of 30) of *A. tepida* did not survive in the culture chamber that contained seawater with the highest calcite saturation state. In contrast, six juvenile foraminifers were found in the same group. The number of individuals that added new chambers is relatively constant between the different groups. On average, 29% of the

individuals of incubated *A. tepida* formed new chambers while 47% of the *H. depressa* specimens precipitated new calcite.

When counting the newly formed chambers of each foraminiferal test, we observed in both species a trend of increasing chamber addition with increasing calcite saturation state (Figure 3). In the Groups 1 and 2 with the lowest Ω values, no considerable difference in chamber addition was apparent. In contrast, in Groups 3 and 4, *A. tepida* produced by up to two times the number of new chambers. The amount of new rows of chambers formed by *H. depressa* was considerably higher in seawater with the highest Ω.

3.3.2 Partition coefficients of Mg and Sr

Since the Mg/Ca ratios of the modified seawater were not exactly the same between the different experiments (Table 1), we plotted the analysed Mg/Ca ratios of calcite (Mg/Ca$_{cc}$) versus the ones of seawater (Mg/Ca$_{sw}$) (Figure 4A). The variation of Mg/Ca$_{cc}$ ratios in *A. tepida* with increasing Mg/Ca$_{sw}$ is negligible which indicates that shell Mg/Ca was not influenced by the small variation of seawater Mg/Ca in this experiment. Conversely, Mg/Ca$_{cc}$ in *H. depressa* increased with increasing Mg/Ca$_{sw}$, suggesting a concentration effect on the Mg incorporation during this experiment. The data, however, do not follow a single through-origin linear fit but show considerable deviations from such a line (Figure 4A).
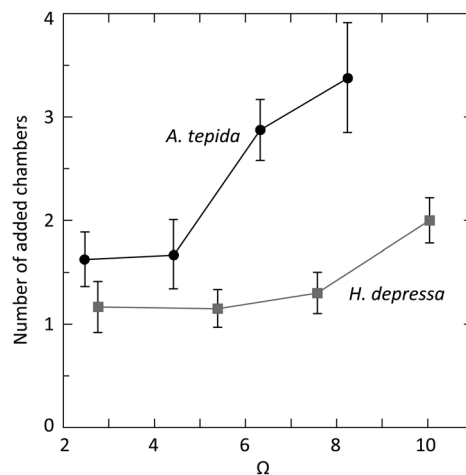

Figure 3. Number of new chambers and new rows of chambers for *A. tepida* and *H. depressa*, respectively, added during two months versus Ω. Error bars are standard errors of the mean. For both foraminifer species, number of newly added chambers and rows tends to increase with increasing Ω.

We calculated the partition coefficients of Mg according to the equation:

$$D_{TE} = \frac{TE/Ca_{Cc}}{TE/Ca_{sw}}$$ (Equation 2)

where TE/Ca$_{cc}$ and TE/Ca$_{sw}$ correspond to the trace element/Ca ratio in the foraminiferal calcite and in seawater, respectively. The observed D$_{Mg}$ values for *H. depressa* range from 2.51*10^{-2} to 2.78*10^{-2} (Figure 4A, Table 2), suggesting that D$_{Mg}$ was altered by the minor variations in Mg/Ca$_{sw}$ and/or by the highly variable Ca concentrations we aimed at with our experiments. Since the Sr/Ca ratios in seawater were not the same between the different experimental solutions -due to the manipulation of the [Ca^{2+}], we calculated the partition coefficients of Sr using equation (2). We observe that D$_{Sr}$ is species-specific with a value of 0.28 for *H. depressa* and 0.16 for *A. tepida* (Figure 4B). The Sr/Ca data fall close to through-origin linear relationships with Sr/Ca$_{sw}$, which means there is no effect of seawater Sr/Ca or Mg/Ca on D$_{Sr}$ for these two benthic species.

However, in order to normalize the absolute trace element/Ca ratios of calcite to the seawater elemental composition, we report our data only in terms of partition coefficients D$_{Mg}$ and D$_{Sr}$ in the following sections.

Table 2. Mg/Ca and Sr/Ca ratios and partition coefficients.

Experiment I *H. depressa*	Group 1	Group 2	Group 3	Group 4
Number of individuals[a]	6	7	5	7
Mg/Ca (mmol/mol)	143.34±11.89	145.56±8.62	151.57±8.34	155.00±11.20
D$_{Mg}$ (x 100)[b]	2.78±0.23	2.61±0.16	2.54±0.14	2.51±0.18
Sr/Ca (mmol/mol)	4.82±0.35	2.66±0.26	1.98±0.10	1.61±0.12
D$_{Sr}$ [b]	0.27±0.02	0.28±0.03	0.31±0.02	0.33±0.02
Experiment II *A. tepida*	Group 1	Group 2	Group 3	Group 4
Number of individuals[a]	6	4	8	7
Mg/Ca (mmol/mol)	2.41±0.51	1.78±0.46	1.60±0.29	3.65±1.95
D$_{Mg}$ (x 1000)[b]	0.47±0.10	0.37±0.08	0.31±0.06	0.70±0.40
Sr/Ca (mmol/mol)	2.56±0.68	1.35±0.19	0.91±0.05	0.78±0.12
D$_{Sr}$ [b]	0.17±0.04	0.16±0.02	0.16±0.01	0.17±0.03

Values are given with ± standard deviations.
[a]Refers to analysed individuals. Not all grown foraminifera were analysed because of limited space available for LA.
[b]Partition coefficients calculated from D$_{TE}$ = (TE/Ca$_{cc}$)/(TE/Ca$_{media}$).

3.3.3 D_{Mg} and D_{Sr} versus calcite saturation state

The experiments on *H. depressa* and *A. tepida* yield decreasing D_{Mg} with increasing Ω for both species (Figures 5A and B). Although it is possible that an exponential function is more appropriate, we chose a linear fit through the data, where D_{Mg} decreases with $4*10^{-4}$ per Ω unit in *H. depressa* (Figure 5A) and with $4*10^{-5}$ in *A. tepida* (Figure 5B). Both regressions are statistically significant at the 5% confidence level (*p* <0.05), obtained from regression analyses. For the *H. depressa* experiment, however, ANOVA (analysis of variance) tests suggests that the overall differences between the population means (i.e. between the different Ω groups) are not significant (*p* >0.05).
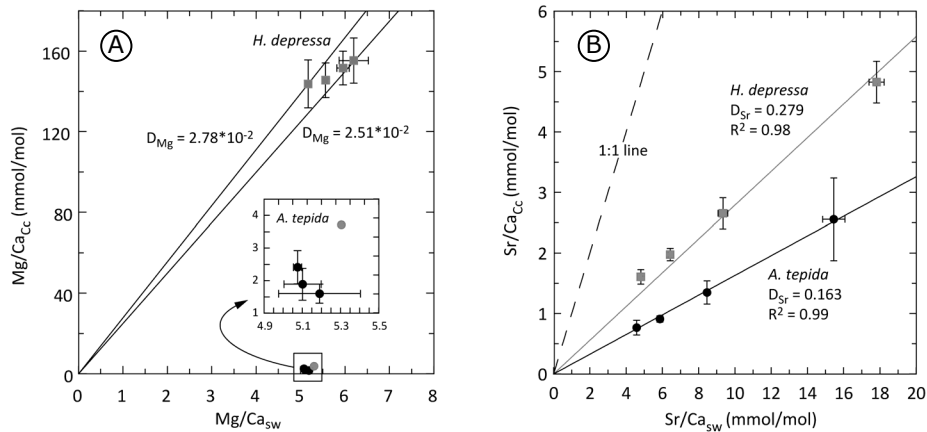
Figure 4. Trace element to Ca ratios in calcite versus seawater for A. magnesium and B. strontium. Grey squares correspond to *H. depressa*, whereas the black dots correspond to *A. tepida*. Lines going through the origin correspond to D values indicated. Error bars (2σ) are based on multiple measurements and variability during the duration of the experiment.

For *A. tepida*, in contrast, the population means are significantly different (*p* = 0.005). The higher D_{Mg} value found in Group 4 of the *A. tepida* experiment is considered an outlier and was therefore excluded from line fitting. Scanning electron microscopy suggests that the foraminiferal tests in this group are affected by inorganic calcite overgrowths enriched in Mg, due to the high calcite saturation of the seawater. The gradient through the remaining three data for *A. tepida* is similar to results from the culture experiments on the planktonic foraminifer *O. universa* by Russell et al. (2004) within the same range of

calcite saturation state, although D_{Mg} values for *O. universa* are by a factor of 3 higher than for *A. tepida* (Figure 5B). In the study of Russell et al. (2004), the dependence of D_{Mg} on pH and [CO$_3^{2-}$] was stronger at lower calcite saturation states, compared to higher saturations where D_{Mg} seemed to be fairly constant (Figure 5B). The D_{Mg} values for *G. bulloides*, as well as, for *G. ruber* (white) from Kısakürek et al. (2008), by comparison, decreased in a similar way with increasing Ω, but the gradients were found to be significantly steeper than those for *A. tepida* and *O. universa* (Figure 5B).

The partition coefficients of strontium for *A. tepida* plotted versus Ω display no correlation (Figure 5C). In contrast, D_{Sr} of *H. depressa* increases linearly with Ω at a gradient of $9*10^{-3}$/Ω unit, with a high statistical significance derived from regression analysis ($p \approx 0.01$). In addition, the overall differences between population means obtained from ANOVA are highly significant ($p <0.001$). The correlation between D_{Sr} and the calcite saturation state for   *H. depressa* is considerably higher than the slight D_{Sr} increase found by Russell et al. (2004) for the planktonic foraminifer *O. universa* (Figure 5C). In our experiment, D_{Sr} for *H. depressa* increased by approximately 23%, whereas D_{Sr} for *O. universa* in the Russell et al. (2004) study increased by only 10% over the same range of Ω changes. Interestingly, the amount of Sr incorporated into tests of *H. depressa* is generally up to two times higher compared to the low-Mg foraminifer species for which the data are shown in Figure 5C.

3.4. Discussion

3.4.1. The effect of [Ca^{2+}] on Mg and Sr incorporation

The influence of [Ca^{2+}] on the Mg incorporation in foraminiferal calcite is rarely investigated. For two benthic high-Mg species, Segev and Erez (2006) have shown that the absolute Ca concentration does not have a clear effect on foraminiferal Mg/Ca ratios. Our results show that this may be true for high-Mg foraminifera, since D_{Mg} of the four experimental solutions are not significantly different ($p >0.05$) (Figure 5A). In contrast, D_{Mg} of the low-Mg species *A. tepida* decreased significantly with increasing [Ca^{2+}], indicating a distinct effect of seawater [Ca^{2+}] on the Mg incorporation into shells of this species (Figure 5B).

The various Ca^{2+} concentrations also resulted in various calcium carbonate saturations of the culturing media. A number of culturing experiments have shown that the Mg incorporation in various planktonic species decreases with higher calcite saturation state (Ω), when Ω is

altered by changing the $[CO_3^{2-}]$ (Kısakürek et al., 2008; Lea et al., 1999; Russell et al., 2004; Figure 5B). Our experiments show that altering Ω by changing the $[Ca^{2+}]$ does result in a similar shift in Mg incorporation in benthic foraminifera. Decreasing D_{Mg} in *A. tepida* with increasing Ω are in line with those reported for the planktonic species (Figure 5B), while the slight D_{Mg} decrease for *H. depressa* is still within reproducibility of replicate measurements and thus displays no significant offset (Figure 5A). The opposite trend (i.e. higher Mg incorporation correlated with higher Ω) has also been reported for various benthic species from field surveys (Elderfield et al., 2006; Healey et al., 2008; Raitzsch et al., 2008).
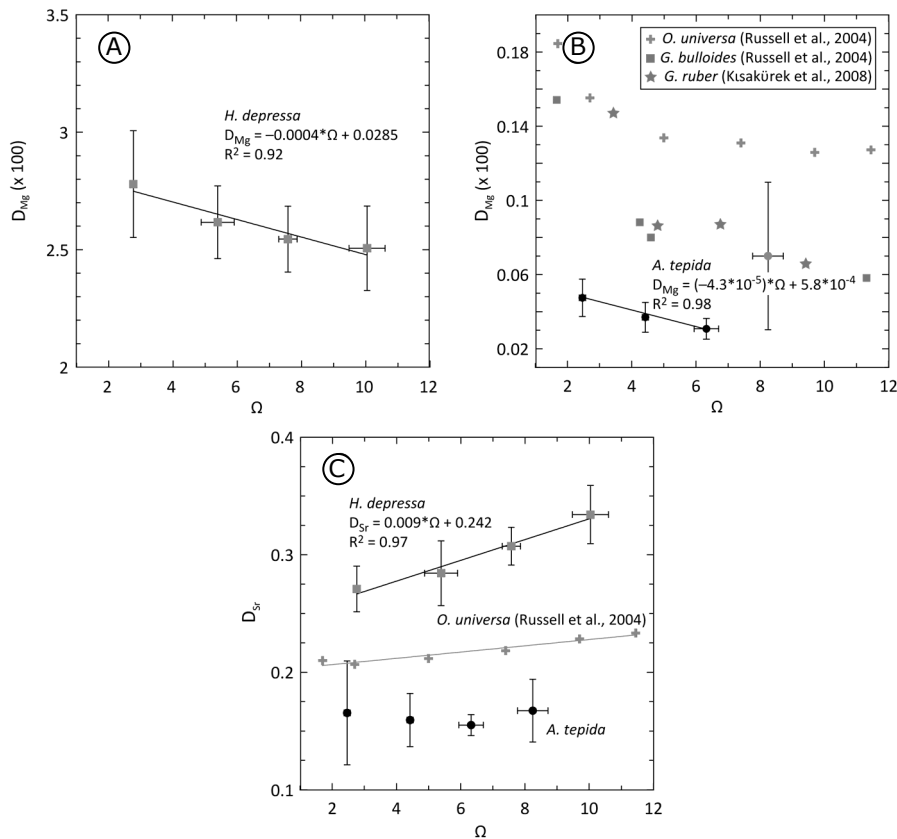
Figure 5. Relation between D_{Mg} of cultured foraminifers and calcite saturation state of the media for A. *Heterostegina depressa* and B. *Ammonia tepida*. Error bars represent standard deviations. The partition coefficients D_{Mg} of both species decrease with increasing Ω. The grey data point in b) is considered an outlier (see text for details). C. Partition coefficient for Sr (D_{Sr}) plotted versus Ω. Black circles correspond to *A. tepida*, grey squares correspond to *H. depressa*. Data are plotted along with strontium data for *O. universa* (crosses) from Russell et al. (2004).

Precipitation rate could possibly be responsible for the observed variations in the Mg incorporation into biogenic carbonates. Laboratory experiments on corals indeed showed that an increase in [Ca^{2+}] had the same effect on the precipitation rate as an increase in [CO$_3^{2-}$] (Gattuso et al., 1998; Langdon et al., 2000). Rather than by precipitation rate, Gaetani and Cohen (2006) showed that seasonal variations of Mg and Sr incorporation in corals are best described by a combination of a temperature effect on element partitioning and variations in the "precipitation efficiency" (the mass fraction of aragonite precipitated from the calcifying fluid). This, in turn, is most likely driven by varying efficiency of the Ca-ATPase enzyme pump or ion channel transport (Gaetani and Cohen, 2006). Like corals, foraminifers possess a variety of such mechanisms to modify the chemistry of the seawater within isolated pools in order to enhance calcification. These physiological processes may be sensitive to ambient environmental conditions (Bentov and Erez, 2006).

The interpretation of benthic foraminiferal Sr fractionation and the use of Sr/Ca ratios in paleoceanography are less straightforward than that of Mg fractionation and Mg/Ca ratios as a proxy for seawater temperature. Variations in the concentration of Sr have been attributed to changes in Sr/Ca of seawater (Lear et al., 2003a), hydrostatic pressure effects (Elderfield et al., 1996), and temperature, and/or carbonate ion concentration (Reichart et al., 2003; Rosenthal et al., 2006). On the other hand, Sr incorporation in planktonic species was shown to increase with increasing pH or [CO$_3^{2-}$] (Dueñas-Bohórquez et al., 2009; Lea et al., 1999; Russell et al., 2004) and increasing growth rate (Kısakürek et al., 2008). The latter is yet difficult to isolate from variations in temperature and salinity since both influence the growth rate of the foraminifer. However, as we changed [Ca^{2+}] between the different solutions, while keeping [Sr^{2+}] constant, we observed nearly perfect through-origin linear correlations between the Sr/Ca ratios in the shells and the media (Figure 4B). The accordant D$_{Sr}$ values of *A. tepida* did not vary with changing Ω, whereas D$_{Sr}$ of *H. depressa* increased significantly with increasing Ω (Figure 5C).

3.4.2. Possible role of Mg/Ca$_{sw}$ on Mg and Sr incorporation

Since the Mg/Ca ratios of seawater between the different solutions displayed some variability (Table 1), we plotted Mg/Ca$_{cc}$ versus Mg/Ca$_{sw}$ to correct for differences in the culture media and thus obtain fractionation factors for Mg in the two investigated species (Figure 4A). For *A. tepida*,

the decrease in Mg/Ca_{cc} with increasing Mg/Ca_{sw} along with the small Mg/Ca_{sw} range from 5.1 to 5.3 is negligible. Therefore, it is likely that the observed variation in Mg/Ca of *A. tepida* is primarily related to the variation of seawater $[Ca^{2+}]$.

Conversely, Mg/Ca_{cc} in *H. depressa* increased with increasing Mg/Ca_{sw} that had a larger range in culture media from 5.2 to 6.2 (Figure 4A). The partition coefficient shows a clear decrease with increasing Mg/Ca_{sw} (Figure 6). This relationship is very similar to the one depicted by Segev and Erez (2006) for *Amphistegina spp.* and by Mucci and Morse (1983) for inorganically precipitated calcites (Figure 6).
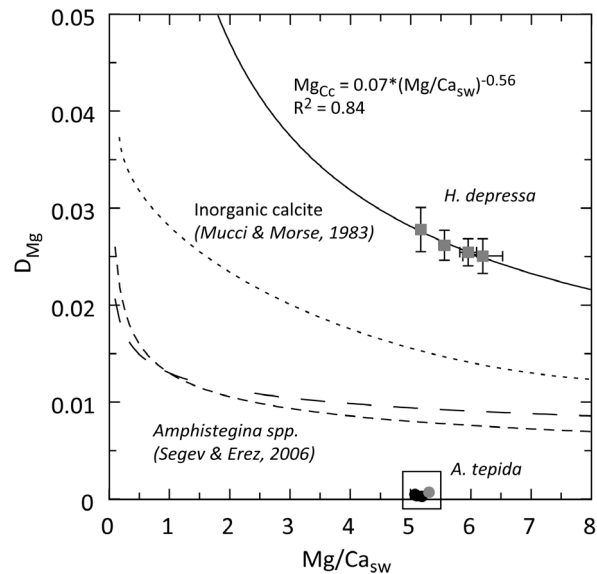
Figure 6. Shell D_{Mg} in relation to the Mg/Ca ratio of seawater. The extrapolated potential fit through the data for *Heterostegina depressa* exhibits a similar shape as found by Segev and Erez (2006) for *Amphistegina lobifera* (widely dashed line) and *A. lessonii* (closely dashed line) and by Mucci and Morse (1983) for inorganic calcite overgrowths (dotted line).

Based on the observation that Mg concentrations in high-Mg foraminifera are comparable to those in inorganic calcite, it seems plausible that the Mg fractionation during calcification in *Amphistegina spp.* and *H. depressa* follows the pure inorganic fractionation.

Mucci and Morse (1983) and Ohde and Kitano (1984) showed that higher Mg/Ca_{cc} and Mg/Ca_{sw} also favours Sr incorporation into $CaCO_3$. This may explain some of the variation in D_{Sr} in our *Heterostegina* experiments, which showed an increase with increasing Mg/Ca_{cc} (Figure

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5C). Such a change in D_{Sr} may be related to a very low ion activity of Mg^{2+} in solutions from which low-Mg carbonate is precipitated, and vice versa (Lorens and Bender, 1980; Morse and Bender, 1990). Alternatively, the Sr^{2+} incorporation is facilitated by the creation of cationic sites larger than Ca^{2+} due to the distortion of the calcite lattice produced by the incorporation of the smaller Mg^{2+} cations (Mucci and Morse, 1983). Both hypotheses could explain why Sr/Ca found in high-Mg calcifying organisms is generally a factor of about two higher compared to low-Mg biogenic carbonates (Morse and Bender, 1990). This is in accordance with the distinct partition coefficients of Sr determined for the high-Mg and low-Mg foraminifera in this study (Figure 4B).

Calculating the theoretical change of D_{Sr} for *H. depressa* using the observations by Mucci and Morse (1983) and Carpenter and Lohmann (1992) from inorganic and various biogenic carbonates, D_{Sr} should have increased by merely 1% as a result of the total Mg/Ca$_{cc}$ increase by 8% (Table 2). We observed a D_{Sr} increase of 23% (Figure 5C), suggesting that only a small portion of the D_{Sr} variation can be attributed to the Mg/Ca$_{cc}$ increase. Consequently, the obtained increase in D_{Sr} of *H. depressa* is largely related to the variable Ca concentrations among the media.

3.4.3. Biomineralization mechanisms

Based on field studies and culturing experiments showing the impact of amongst others temperature, salinity, Ω and seawater elemental concentrations on trace element incorporation, and stable isotope fractionation, several conceptual models have been developed to explain these relationships. These models are commonly based on a foraminiferal calcification pathway starting with seawater vacuolisation (De Nooijer et al., 2009a; Erez, 2003) and subsequent modifications resulting in the separate production of intracellular pools containing calcium and carbonate (Bentov and Erez, 2006; Erez, 2003).

The low Mg/Ca ratios in calcite of most species implies that during formation of the internal Ca pool, these species actively discriminate against Mg so that the Ca pool has a Mg/Ca ratio considerably lower than that of seawater. Although not identified directly, this could be caused by selective removal of Mg^{2+} from the Ca reservoir or by Ca-pumps actively pumping Ca^{2+} from vacuolised seawater into the Ca reservoir. Both of these mechanisms can explain how foraminifers produce a Ca pool with a low Mg/Ca ratio. It has been shown that the Mg isotopes in low-Mg foraminiferal calcite are strongly depleted in comparison to those of inorganically precipitated calcite (Pogge von Strandmann, 2008). This

implies that the physiological mechanism reducing Mg/Ca ratios in the intracellular Ca pool selectively let in isotopically lighter Mg ions. This, in turn, suggests that these species transport Ca using seawater vacuolisation, since active Mg^{2+} removal would more likely result in an isotopically enriched Mg signature in the intracellular Ca pool. In any version of a calcification model, selective sequestration of Mg^{2+} by organic compounds may be involved in reducing the Mg/Ca ratio of the internal calcification fluid (Bentov and Erez, 2006).

The high Mg/Ca ratios in *H. depressa* may indicate that the calcification strategy in this species is fundamentally different from that of *A. tepida*, which may in turn explain the atypical response of the former group to altered Ω. The low partition coefficient for Mg of *A. tepida* (as in most foraminiferal species; Blackmon and Todd, 1959; Erez et al., 2003) compared to those for most other trace elements, suggests that foraminifers adopt a physiological mechanism to discriminate between Ca^{2+} and Mg^{2+} during production of the internal Ca pool. Some species, however, produce calcite that has a Mg concentration comparable to calcium carbonates inorganically precipitated from media with Mg/Ca ratios comparable to seawater (Katz, 1973; Oomori et al., 1987). This suggests that these species do not have this discriminating mechanism and that they solely rely on increasing the pH at the site of calcification (Erez, 2003; Zeebe and Sanyal, 2002), which is also found in high-Mg species (De Nooijer et al., 2009b; Zeebe and Sanyal, 2002). This also implies that Mg/Ca ratios in high-Mg taxa are directly related to the Mg/Ca ratio in seawater, rather than to absolute concentrations of Ca and Mg, which explains the similar Mg/Ca ratios in *H. depressa* in our experiment.

3.5. Conclusions

Culturing experiments show that the Mg incorporation into shells of *H.depressa* and *A. tepida* decreased with increasing calcium concentration and thereby calcite saturation state of the seawater (Ω). The decrease in D_{Mg} of *A. tepida* at a gradient of $4.3*10^{-5}$ per Ω unit is statistically significant, whereas the one of *H. depressa* is insignificant. The slight decrease in D_{Mg} with increasing Ω observed in the *H. depressa* experiment, however, may rather be related to minor variations in the Mg/Ca ratio between the different groups.

The observed strong variability of Mg/Ca in *A. tepida* suggests a mechanism actively depleting Mg and/or enriching Ca in the intracellular calcification pool. The efficiency of this mechanism may depend on the ambient seawater conditions. The insignificant change in D_{Mg} of *H.*

depressa between the different groups suggests that this high-Mg species possibly lacks such a mechanism to fundamentally alter the internal Ca and/or Mg concentrations, using a different calcification pathway (e.g., increasing pH by endosymbiotic activity).

The influence of Ω on the Sr incorporation was different between both species, displaying no effect in *A. tepida* and a highly significant effect in *H. depressa* with a sensitivity of 0.009/Ω unit. The D$_{Sr}$ values of both species are shown to be largely independent from the Sr/Ca ratio of seawater, suggesting that Sr/Ca ratios in fossil shells of these shallow-water benthic foraminifera may be used to reconstruct past variations in Sr/Ca of seawater.

Acknowledgements

Chapter 4

Impact of carbonate ion concentration and calcite saturation state on Mg and Sr incorporation in cultured benthic foraminifera

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Abstract

Laboratory culture experiments were carried out to determine the effect of the carbonate ion concentration of seawater ($[CO_3^{2-}]$), and thereby calcite saturation state (Ω), on Mg and Sr incorporation into the calcite of two species of shallow-water benthic foraminifera: *Ammonia tepida* and *Heterostegina depressa*. An increase in the $[CO_3^{2-}]$ of seawater and thus Ω, appears to promote incorporation of Mg and Sr into the calcitic tests of both species. The response of Mg and Sr incorporation in both species is comparable, even though the Mg/Ca ratio is ~70 times higher in *H. depressa* compared to *A. tepida*. We thus show that at increasing $[CO_3^{2-}]$ the calcification processes are affected in similar ways by the same seawater chemical parameter, although the magnitude of this effect varies between species. These results differ from another series of experiments in which the effect of the Ca concentration on Mg and Sr incorporation in the same species was investigated. The $[CO_3^{2-}]$ has a minor and contrasting influence on Mg incorporation compared to the effect of $[Ca^{2+}]$, even though they both affect the calcite saturation state of the seawater (Ω). This implies that Ω is not directly responsible for the altered Mg incorporation into the calcite of *A. tepida* and *H. depressa.* The combined results of these two studies indicate that ions (Ca^{2+} and CO_3^{2-}) involved in biomineralization are taken up through separate transport mechanisms. The similar response of Mg and Sr incorporation in this study suggests that only differences in the Ca^{2+} transport mechanism affect divalent cation uptake. The two ion transport processes are thus apparently independent.

4.1. Introduction

Foraminifera constitute one of the most important groups of calcifying organisms in the ocean, producing between 32-80% of the annual calcium carbonate (CaCO$_3$) flux deposited in the deep ocean (Schiebel, 2002). The ongoing increase of atmospheric greenhouse gasses results in increasing dissolution of CO$_2$ in the seawater and subsequent acidification of the oceans (Zeebe and Wolf-Gladrow, 2005). Ocean acidification results in an altered carbonate chemistry of seawater decreasing the calcite saturation state (Ω). A decline in Ω may have important consequences for reproduction, growth and calcification rates of foraminifera (Wolf-Gladrow et al., 1999b; Zeebe and Wolf-Gladrow, 2005). Although a number of studies have explored the effects of a decreasing pH on coccolithophores (e.g. Riebesell et al., 2000; Langer et al., 2009), scleractinian corals (Gattuso et al., 1998; Langdon et al., 2000) and echinoderms (Clark et al., 2009), the individual impact of the calcium and carbonate concentrations in seawater ([Ca^{2+}] and [CO$_3^{2-}$], respectively), parameters that control Ω, on benthic foraminiferal calcification is rarely investigated.

The seawater saturation state with respect to calcite determines the kinetics of formation or dissolution of CaCO$_3$ and is described by: Ω = [Ca^{2+}]*[CO$_3^{2-}$]/K$_{sp}$, where K$_{sp}$ represents the solubility product at the *in situ* conditions of temperature, salinity and pressure for the CaCO$_3$-phase under consideration (Zeebe and Wolf-Gladrow, 2005). Besides the effect of [CO$_3^{2-}$], and therefore Ω, on the biology of foraminifera, its consequences for the paleoceanographic application of foraminiferal Mg/Ca and Sr/Ca ratios has been a key issue in several studies (Lea et al., 1999; Russell et al., 2004; Mortyn et al., 2005; Rosenthal et al., 2006; Elderfield et al., 2006). These studies showed that foraminifera display various sensitivities and occasionally even opposite trends in their trace and minor element composition in response to altered seawater carbonate chemistry (Russell et al., 2004; Rosenthal et al., 2006; Rathmann and Kuhnert., 2008; Raitzsch et al., 2008; Dueñas-Bohórquez et al., 2009).

The aim of this study is to determine the effect of seawater [CO$_3^{2-}$] and thus Ω, on Mg and Sr incorporation into the calcitic tests of the shallow-water symbiont-barren foraminifer *Ammonia tepida* and the tropical foraminifer *Heterostegina depressa*, associated with frustule-less diatom endosymbionts. These two benthic species were selected because of their contrasting calcification strategies: while *A. tepida* precipitates calcite with a very low Mg content, *H. depressa* builds a test with Mg concentrations ~70 times higher than that of low-Mg species. In addition to providing an improved calibration for foraminiferal Mg/Ca and Sr/Ca

ratios, the presented results improve our understanding of foraminiferal calcification pathways and help to predict their response to ongoing ocean acidification.

4.2. Methodology

4.2.1. Collection and culturing of foraminifera

Specimens of *A. tepida* were collected from the mudflats of the Dutch Wadden Sea. Specimens of *H. depressa* were collected from tropical aquaria at Burgers' Zoo (Arnhem), the Netherlands. Stocks of both species were kept in thermostatically controlled incubators (Aqualytic) prior to the experiments. Sediment containing specimens of *A. tepida* was kept in aquaria at a constant temperature (18 $\pm$ 0.5°C) and salinity (25). Individuals of *H. depressa* were kept in aquaria at a temperature of 24 $\pm$ 0.5°C and salinity of 35 under a 12-hour light/12-hour dark cycle.

Individuals of *A. tepida* (size fraction 125-150 µm) containing yellow colored protoplasm were isolated from the sediment and placed in seawater (salinity 35) with calcein dissolved at a concentration of 7 mg/l. Specimens of *H. depressa* (size range 300-600 µm) were isolated and placed in a seawater/calcein mixture with the same calcein concentration. Individuals calcified and incorporated the fluorescent calcite marker to label newly formed chambers (Bernhard et al., 2004) during two to three weeks. After this incubation period, individuals of both species with marked chambers were selected for the culturing experiments (Figure 1). Specimens were placed in 30 ml culture chambers (Figure 2A), which consist of a polyacetal plastic bottom and lid that are separated by a diaphanous plexiglas ring (Figure 2B). At the bottom of the culture chamber a 0.2 µm filter (cellulose acetate) was placed, on top of which a thin layer of artificial sediment (SiO_2) was added. Before starting the experiments, a small amount of freeze-dried mixture of microalgae/diatom (*Dunaliella salina* and *Phaeodactylum tricornutum*) was provided as a food source for *A. tepida*. Each culture chamber contained 30 to 35 specimens of *A. tepida* or *H. depressa*.

Chambers were connected to a continuous seawater-flow system with 3 l gas-tight reservoirs (Tedlar ®) that contained seawater with modified carbonate chemistry (Figure 2). These gas-tight bags were used to minimize CO_2 exchange between the chemically modified seawater and the atmosphere. The control solution (unmodified seawater) was kept in a 1 l glass bottle reservoir open to the atmosphere (Figure 2).

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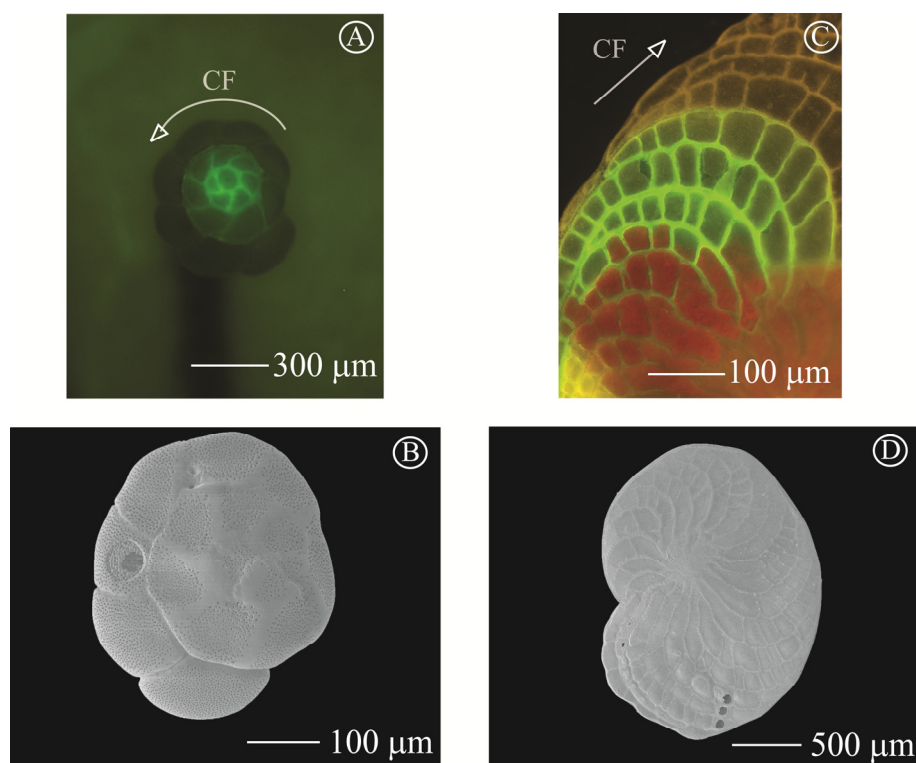
Figure 1. A and B. Shallow-water symbiont-barren benthic foraminifer *Ammonia tepida*. C and D. Tropical symbiont-bearing benthic foraminifer *Hetergostegina depressa*. Fluorescent calcite corresponds to chambers that were built in the seawater-calcein solution prior experimentation. Non-fluorescent calcite corresponds to chambers that grew during the experiments. CF refers to direction of chamber formation. Laser ablation craters are shown in B and D.

The modified seawater solution was discarded after flowing through the culture chambers. Before consuming all modified seawater from the gas-tight bags, the remaining solution was discarded and replaced; this was done every two weeks for the complete incubation period. By limiting the time the solutions were stored in the gas-tight bags, we avoided changes in the carbonate chemistry. During the culturing period, the whole system was kept in incubators at 18 ± 0.5°C for *A. tepida* and 24 ± 0.5°C for *H. depressa*. These temperatures were chosen in accordance with the natural conditions at which these species were collected. Salinity was kept constant at 35 ± 0.3 in all experiments.

The culturing experiments ran for two months after which individuals with newly formed (i.e. non-labeled) chambers (Figure 1) were

collected using an inverted fluorescence microscope. Specimens were then cleaned and prepared for LA-ICP-MS analyses (see section 2.3).
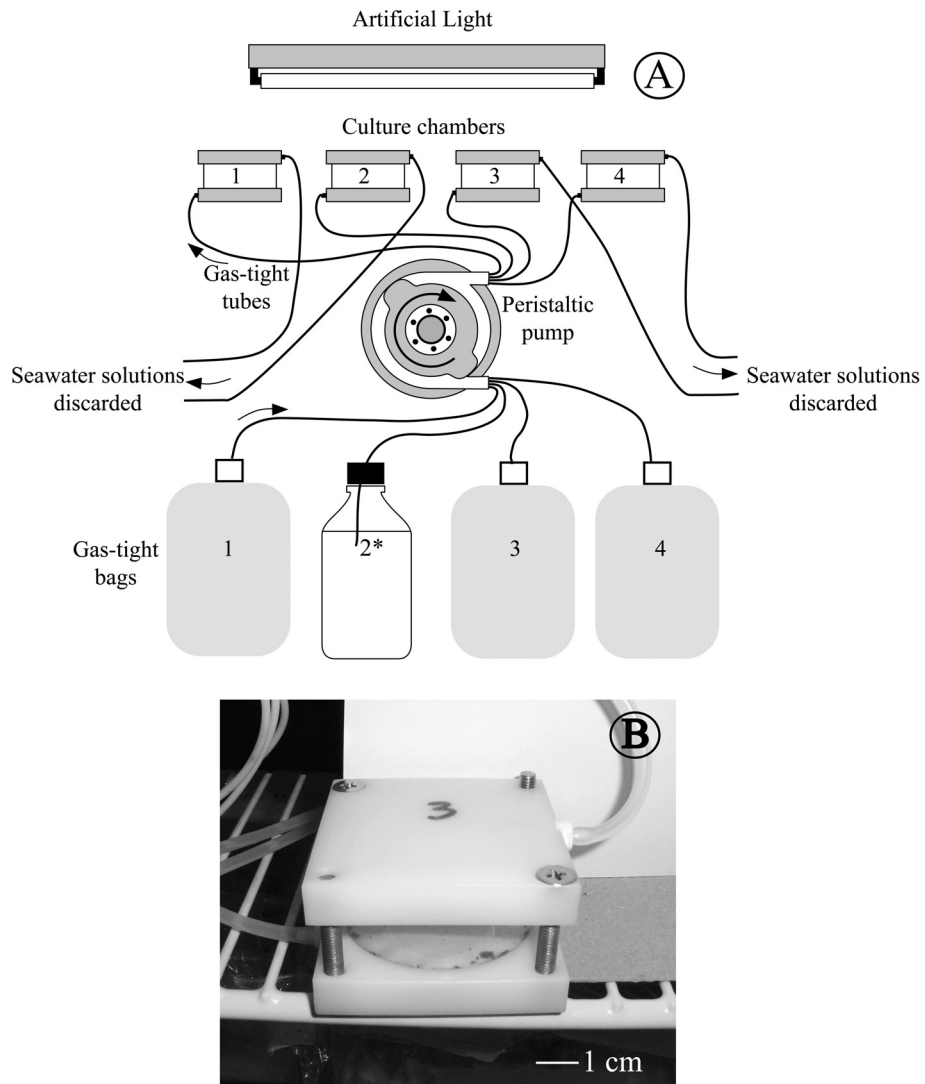
Figure 2. A. Scheme of the seawater flow-through system. Numbers in the reservoirs refer to seawater solutions with different carbonate chemistry. Asterisk (*) indicates unmodified seawater; B. Culture chamber.

4.2.2. Carbonate chemistry of the culture solutions

Culture solutions with four different Ω values were prepared (Table 1). Carbonate parameters of all solutions were calculated using the CO$_2$sys program (version 01.05; Lewis and Wallace, 1998) and sub-sampled twice in the first week and once every following two weeks to monitor salinity, total alkalinity (TA) and dissolved inorganic carbon (DIC) (Table 1). In order to reach target Ω values, we varied seawater [CO$_3^{2-}$] by adding NaOH (1 M) or HCl (1 M) while keeping DIC constant. This approach is complementary to that used by Raitzsch et al. (2009) which varied Ω by changing the [Ca^{2+}].

Table 1. Experimental conditions[a].

| Experiment No.	Salinity	Alkalinity (µmol/kg)	DIC (µmol/kg)	Mean [CO$_3^{2-}$] (µmol/kg)	Ωc[b]
A. tepida					
1	35 ± 0.1	2236 ± 5	2074 ± 36	124 ± 22	3 ± 0.5
2	35 ± 0.3	2340 ± 28	2137 ± 74	158 ± 43	4 ± 0.8
3	35 ± 0.1	2500 ± 11	2076 ± 40	298 ± 31	7 ± 0.7
4	35 ± 0.1	2700 ± 12	2084 ± 26	432 ± 22	10 ± 0.5
H. depressa					
1	35 ± 0.1	2250 ± 5	2080 ± 25	130 ± 13	3 ± 0.3
2	35 ± 0.1	2500 ± 40	2074 ± 25	290 ± 37	7 ± 0.9
3	35 ± 0.1	2550 ± 74	2098 ± 29	324 ± 63	8 ± 1.5
4	35 ± 0.1	2800 ± 40	2114 ± 48	465 ± 34	11 ± 0.8

Values are given with ± standard deviations.
[a]All experiments were carried out at T= 18 ± 0.5°C for *A. tepida* and, 24 ± 0.5°C for *H. depressa*. Mean [CO$_3^{2-}$], *p*CO$_2$ and Ωc were calculated from alkalinity and DIC measurements using the program CO$_2$sys (Lewis and Wallace, 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). Experiment 1 refers to Ω/2, experiment 2 = natural Ω, experiment 3 = 1.5*Ω and, experiment 4 = 2*Ω.
[b]Ωc refers to saturation state for calcite.

4.2.3. Sample preparation and analysis

Specimens with newly formed chambers were placed in sodium hypochlorite (NaClO, 5%) for 10 minutes in order to remove organic material. The specimens were then rinsed three times with deionised water and dried in petri dishes at room temperature. Magnesium and strontium were measured with laser ablation inductively coupled plasma-mass spectrometry (LA-ICP-MS). This micro-analytical technique allows the analysis of elemental concentrations in individual chambers (Reichart et al., 2003). Individual foraminiferal chambers were ablated using a 193 nm laser (GeoLas 200Q Excimer) in a helium flushed ablation chamber
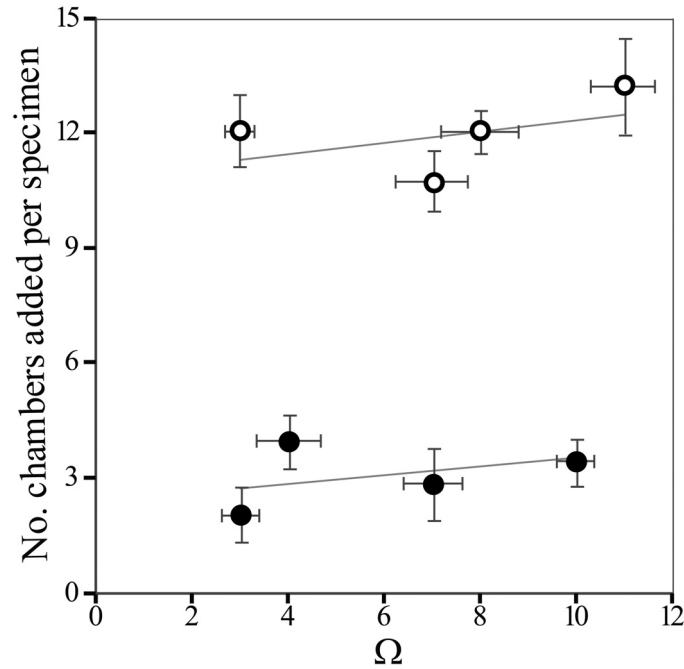
Figure 3. Growth rates in terms of number of chamber added per specimen analysed for each culture experiment. Closed circles correspond to *Ammonia tepida*; open circles correspond to *Heterostegina depressa*.

coupled to a quadrupole ICP-MS (Micromass Platform). Pulse repetition rate was set at 6 Hz with an energy density at the sample surface of 1 J/cm^2. The beam diameter was adjusted to 60 and 80 µm for *A. tepida* and *H. depressa*, respectively, and the ablated calcite was analysed with respect to time. The Mg and Sr concentrations were determined using ^{24}Mg, ^{26}Mg and ^{88}Sr isotopes respectively, using their natural relative abundances. Calibration was performed using a silicate standard NIST 610 (Pearce et al., 1997) and ^{44}Ca as an internal standard. The use of calcium as an internal standard is ideal because this element is present at a constant concentration of 40% in calcite. Concentrations are reported here as Mg/Ca and Sr/Ca ratios and correspond to the average value of single measurements during a laser ablation ICP-MS analysis.

The NIST 610 was ablated 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 employed. This standard is an uncommonly homogeneous calcite crystal (Icelandspar) that was measured using four different ablation energy densities (Figures

B1 and B2). Results show that analysed Mg and Sr values do not significantly vary when different energy densities were used to ablate the calcite crystal. Based on repetitive analyses of the carbonate standard throughout the analytical period, the relative precision for Mg and Sr was approximately 6 and 4%, respectively, for *A. tepida* and, 3.5 and 2.5%, respectively, for *H. depressa*. Simultaneous monitoring of ^{42}Ca, ^{43}Ca, and ^{44}Ca showed isotopic ratios that were 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. The intervals of the acquired data used to calculate concentrations were selected avoiding sections with high ^{27}Al and/or ^{208}Pb counts. This ensures that an unidentified phase does not introduce errors in the trace and minor metal analyses, although foraminiferal chambers added during the experiments were never in contact with natural sediments as a source for contamination. Analysis of variance (ANOVA) was used in order to determine the quality of a regression model that predicts Mg/Ca and Sr/Ca variability under the analysed conditions.

Since there is no ontogenetic effect on Mg incorporation in *A. tepida* (de Nooijer et al., in prep.), the positions of the different chambers (F, F-1, F-2, etc.) were not accounted for in our analysis. In the case of *H. depressa*, different rows of chambers were analysed (Figure 1B).

4.3. Results and Discussion

4.3.1. Survival and growth rates

Survival rate was measured in terms of number of individuals alive at the end of the culture experiments (Table 2). In all experiments, the numbers of *A. tepida* increased during the experimental period, indicating reproduction during these two months. This implies that the experimental setup does not hinder the functioning of *A. tepida* and we, therefore, exclude the possibility that sub-optimal culture conditions affected calcification. Even though a large amount of specimens of *A. tepida* was born during the culture period, these new foraminifera were too small to allow accurate laser-ablation measurements. The minor element data shown here is therefore based on adult specimens that added new chambers during the experiment. All specimens of *H. depressa* were found alive after the two-month experimental period. These living specimens were identified by the presence of cytoplasm which had a bright red colour under the fluorescence microscope. However, only ~30% of the

individuals added new chambers to their test, and no reproduction was observed (Table 2). Although culture conditions may not have been optimal for this species, the large number of newly added chambers by the specimens that did grow indicates that those organisms were not seriously hindered by the artificial conditions.

Growth rates for both species (expressed here in terms of number of added chambers) slightly increase at higher Ω values (Table 2; Figure 3). This observation confirms results from previous culture studies using the same species (Raitzsch et al., 2009) and indicates that Ω has an effect on growth rate. Lorens (1981) and Lopez et al. (2008) also observed an increase in the rate of inorganic calcite precipitation at higher Ω values. This suggests that the extent to which biogenic calcite precipitation changes with environmental parameters may largely depend on thermodynamic factors.

Table 2. Survival and growth rates.

Experiment No.	No. specimens at the beginning	No. living specimens at the end	No. specimens analysed	Total No. chambers added	No. chambers added per individual
A. tepida					
1	31	82	9	17	2.0
2	30	21 + juveniles (>50)	8	32	4.0
3	34	34 + juveniles (>50)	10	28	2.8
4	36	84	11	37	3.4
H. depressa					
1	30	30	9	109	12.0
2	31	31	11	118	10.7
3	35	35	12	142	12.0
4	30	30	5	66	13.2

4.3.2. Effect of [CO$_3^{2-}$] and the calcite saturation state on Mg/Ca ratios

We observe a slight increase in Mg/Ca ratios at higher [CO$_3^{2-}$] (Ω) values for both *A. tepida* and *H. depressa* (Table 3; see Appendix, Tables B1 and B2; Figure 4). However, the increase shown in Mg incorporation is not statistically significant ($p > 0.05$) within the [CO$_3^{2-}$] range studied for either species (124 to 465 µmol/kg; Table 1; Figure 4A and C).

Table 3. Mean Mg/Ca and Sr/Ca ratios and mean D_{Mg} and D_{Sr}.

Experiment No.	n[a]	Mg/Ca (mmol/mol)	Sr/Ca (mmol/mol)	$D_{Mg} * 10^{-3}$	D_{Sr}
A. tepida					
1	9	1.63± 0.06	1.47 ± 0.04	0.315 ± 0.08	0.181 ± 0.013
2	8	1.69 ± 0.03	1.35 ± 0.03	0.320 ± 0.10	0.161 ± 0.012
3	10	1.87 ± 0.08	1.39 ± 0.04	0.365 ± 0.13	0.171 ± 0.010
4	11	2.00 ± 0.07	1.50 ± 0.04	0.393 ± 0.14	0.190 ± 0.021
H. depressa				$D_{Mg} * 10^{-1}$	
1	9	135 ± 4	2.50 ± 0.07	0.264 ± 0.02	0.305 ± 0.020
2	11	132 ± 3	2.56 ± 0.06	0.250 ± 0.02	0.310 ± 0.025
3	12	134 ± 3	2.42 ± 0.04	0.270 ± 0.02	0.300 ± 0.024
4	5	144 ± 6	2.54 ± 0.10	0.283 ± 0.02	0.315 ± 0.037

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

The trend observed in Mg/Ca ratios to variable $[CO_3^{2-}]$ (and thus variable Ω) is comparable to field studies using the deep-sea benthic species *Cibicidoides wuellerstorfi* and *Cibicidoides mundulus* (Yu and Elderfield., 2008; Raitzsch et al., 2008) (Figure 5A). This suggests that different types of benthic foraminifera (e.g. symbiont-bearing, symbiont-barren shallow and deep water species) have a similar response in Mg incorporation to variable $[CO_3^{2-}]$ and thus Ω. A comparison between our Mg/Ca results and those from studies on other benthic species (Yu and Elderfield, 2008; Raitzsch et al., 2008), suggests that despite similarities in trends, sensitivity of Mg/Ca ratios to changes in $[CO_3^{2-}]$ is species-specific (Figure 5A).

The Mg/Ca-$[CO_3^{2-}]$ relationships presented in this study are opposite to those found in the planktonic species *Globigerina bulloides* and *Orbulina universa*, where Mg/Ca ratios decrease with increasing $[CO_3^{2-}]$ (Lea et al., 1999; Russell, et al., 2004) (Figure 5A). From inorganic calcite precipitation studies (Mucci and Morse, 1983; Lopez et al., 2009), it is known that Ω (variable $[CO_3^{2-}]$) does not considerably influence the amount of Mg incorporated into calcite (<2%) within an Ω range of 2 to 11. Our results suggest a small response of *A. tepida* and *H. depressa* in the Mg/Ca ratios of only 3 and 1%, respectively (Table 1), which is in the same range as the inorganic calcite results. Bentov and Erez (2006) suggested that theoretically higher internal saturation values, modified by the organism itself, might allow the precipitation of a (less stable) high Mg-calcite. This agrees well with the results obtained in the present and previous studies on benthic foraminifera (Figure 5A), indicating that the Mg incorporation into the tests of benthic species at variable Ω values

(variable $[CO_3^{2-}]$) is to a large extent controlled by thermodynamic processes.
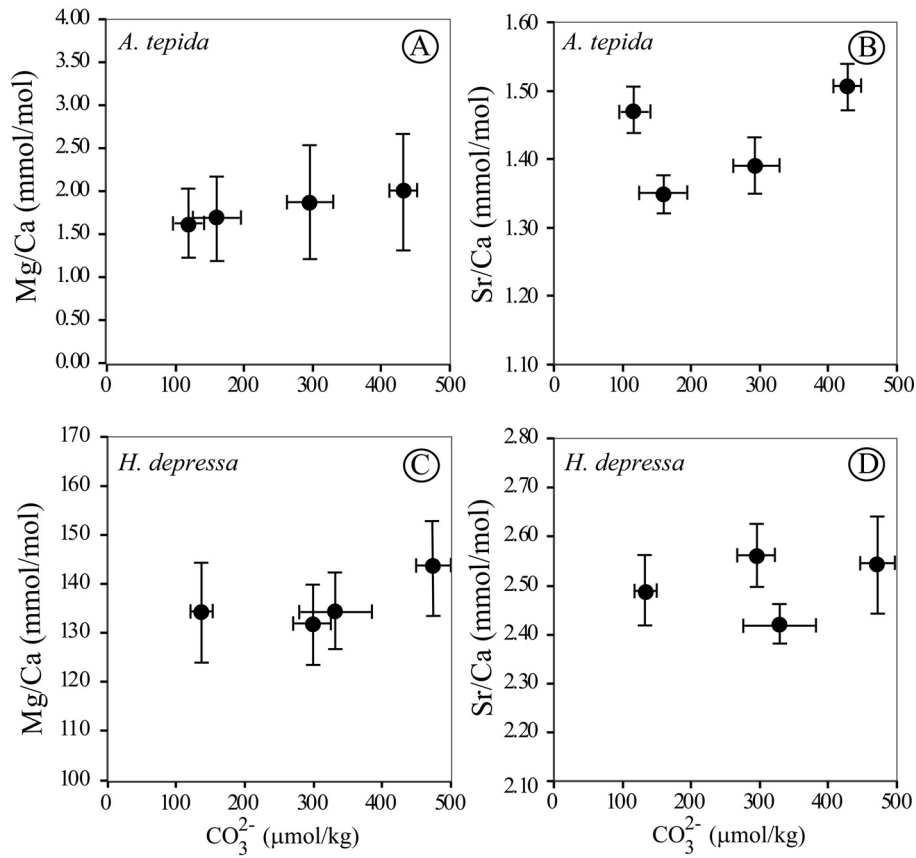
Figure 4. Mg/Ca and Sr/Ca ratios versus carbonate concentration ($[CO_3^{2-}]$). A and B, Mg/Ca and Sr/Ca ratios, respectively, of *Ammonia tepida*; C and D, Mg/Ca and Sr/Ca ratios, respectively, of *Heterostegina depressa*. Error bars are standard deviations of mean values.

4.3.3. Effect of $[CO_3^{2-}]$ and the calcite saturation state on Sr/Ca ratios

We observe a minor increase in Sr/Ca ratios of *A. tepida* and *H. depressa* at higher $[CO_3^{2-}]$ values (higher Ω) (Table 3; Figure 4B and D). The increase in Sr/Ca ratios of *A. tepida* is significant ($p < 0.05$) within the $[CO_3^{2-}]$ interval 158 to 432 µmol/kg (Figure 4B), while the trend in Sr/Ca values of *H. depressa* is not significant ($p > 0.05$; Figure 4D). When comparing these results with changes in Sr/Ca ratios in planktonic

foraminifera caused by variable [CO$_3$$^{2-}$] (Russell et al., 2004; Dueñas-Bohórquez et al., 2009), the results are similar (Figure 5B). Rosenthal et al. (2006) obtained similar results from core-top samples of the aragonitic foraminifer *Hoeglundina elegans*. This combination of results suggests that the mechanisms involved in the incorporation of Sr into foraminiferal CaCO$_3$ respond in a similar way to changes in the carbonate ion concentration for a large variety of species.

In inorganic and biogenic calcite, the Sr/Ca ratio is known to increase with growth rate (Nehrke et al., 2007; Kısakürek et al., 2008), and higher [CO$_3$$^{2-}$] increases foraminiferal shell weight and possibly calcification rates (Bijma et al., 1999). Our results show that growth rate of both benthic foraminiferal species (expressed here as the number of new chambers added during the experiments) slightly increases with increasing [CO$_3$$^{2-}$] (Table 2; Figure 3). Consequently, the minor increase in Sr/Ca ratios at higher [CO$_3$$^{2-}$] observed in both benthic species is possibly linked to growth rates.

4.3.4. Effect of the calcite saturation state on D_{Mg} and D_{Sr}

The effect of Ω on Mg/Ca and Sr/Ca ratios of *A. tepida* and *H. depressa* has been analysed in terms of the empirical partition coefficient of these minor elements (D_{ME}). We consider D_{Mg} and D_{Sr} in order to compare two sets of culture experiments which have seawater solutions with slightly different minor element compositions (Raitzsch et al., 2009 and present study). By expressing the results as a function of D, we circumvent the possible effect that differences in ME/Ca in seawater might have on Mg/Ca and Sr/Ca ratios.

The partition coefficient is defined by [ME]/[Ca]$_{calcite}$ = D * [ME]/[Ca]$_{seawater}$, where [ME] indicates the actual concentration of the minor element in calcite and seawater respectively (Mucci and Morse, 1990). The partition coefficients of Mg and Sr for both species slightly increase with increasing Ω ([CO$_3$$^{2-}$]) values (Table 3; Figure 6).

The effect of Ω (variable [CO$_3$$^{2-}$]$_{sw}$) on D_{Mg} and D_{Sr} of *A. tepida* is opposite to the effect this parameter has on the same species when [Ca^{2+}]$_{sw}$ varies (Raitzsch et al., 2009; Figure 6A and B). Dissard et al. (2009c) observed an increase in D_{Mg} and D_{Sr} at higher Ω values for *A. tepida* which agrees with the results presented here (Figure 6A and B). However, the responses observed in D_{Mg} and D_{Sr} by Dissard et al. (2009c) are larger (steeper slopes) than calculated here, possibly as a result of additional changes in total alkalinity (TA), dissolved inorganic carbon (DIC), salinity, [CO$_3$$^{2-}$] or [Ca^{2+}].
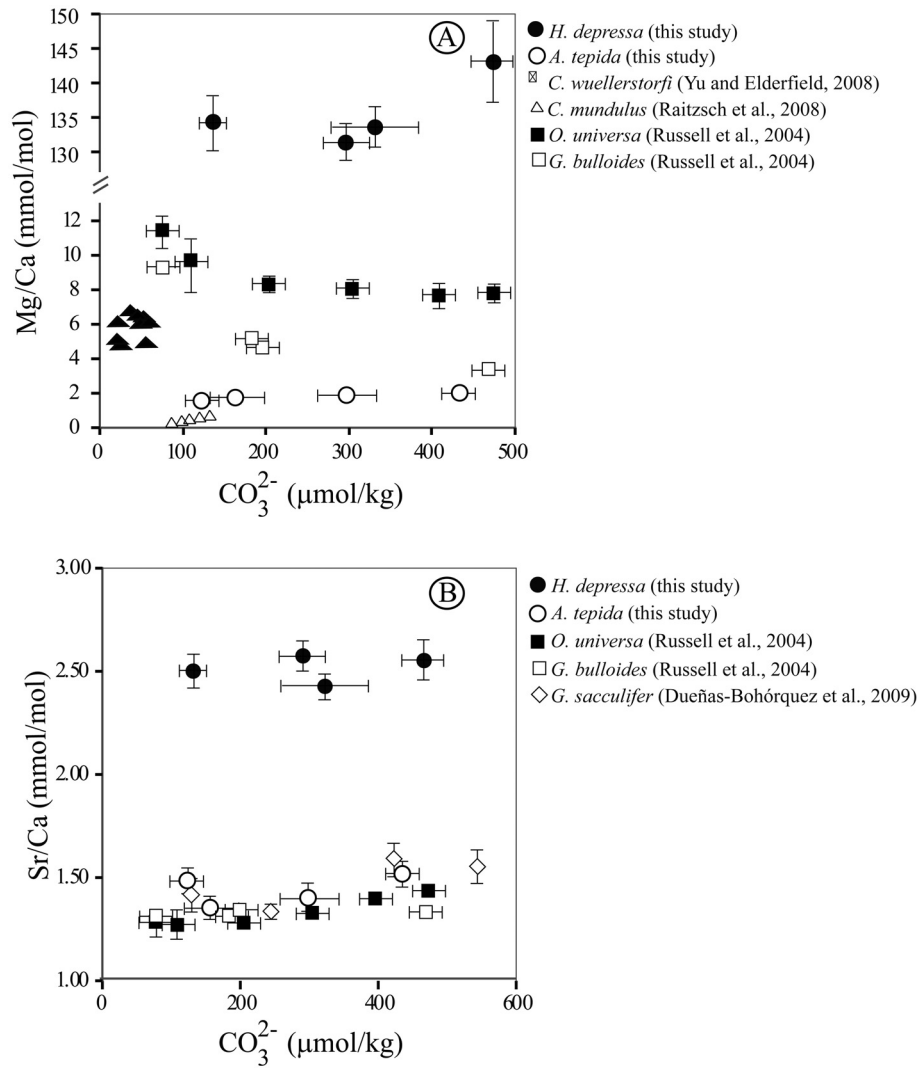
Figure 5. A. Foraminiferal Mg/Ca and B. Sr/Ca ratios vs. $[CO_3^{2-}]$. Data from Raitzsch et al. (2008) correspond to ΔMg/Ca which is defined as the variation in Mg/Ca ratios only due to variable $[CO_3^{2-}]$ seawater. The Mg/Ca and Sr/Ca ratios correspond to a temperature of 24ºC for all benthic species except for Mg/Ca and Sr/Ca ratios of *Ammonia tepida* which correspond to a temperature of 18ºC.

In the case of D$_{Mg}$ of *H. depressa*, opposite responses are observed depending on the Ω component that varies: [CO$_3^{2-}$]$_{sw}$ (present study) or [Ca^{2+}]$_{sw}$ (Raitzsch et al., 2009; Figure 6C). Part of the response observed in D$_{Mg}$ of *H. depressa* for the experiments in which [Ca^{2+}]$_{sw}$ was varied might, however, be due to significant changes in the Mg/Ca ratios of the experimental solutions (Raitzsch et al., 2009). The response of D$_{Sr}$ of *H. depressa* to changes in Ω is similar in both studies (present study and Raitzsch et al., 2009). However, the magnitude of the effect of [CO$_3^{2-}$]$_{sw}$ is smaller than the one observed for [Ca^{2+}]$_{sw}$.
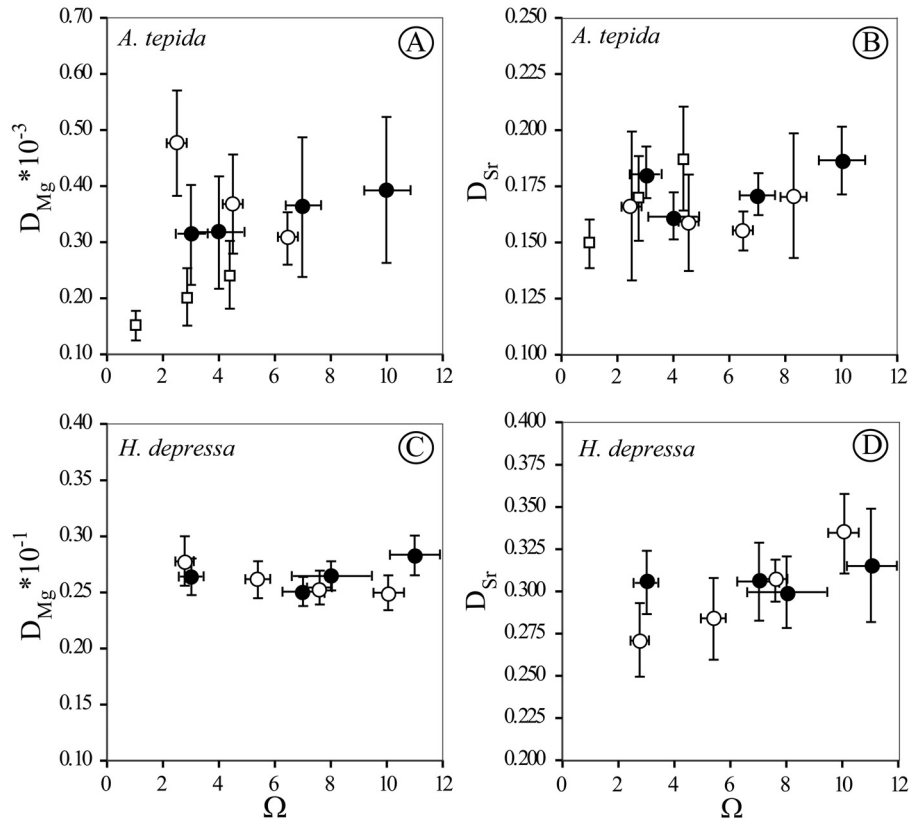
Figure 6. Partition coefficients of Mg and Sr of *Ammonia tepida* (A and B) and *Heterostegina depressa* (C and D) vs. the calcite saturation state of the seawater (Ω) under two different seawater chemical conditions: 1. variable Ω due to changes in [CO$_3^{2-}$] seawater (closed circles, present study) and, 2. variable Ω due to changes in [Ca^{2+}] seawater (open circles, Raitzsch et al., 2009). Open squares correspond to data from Dissard et al. (2009) at a constant temperature of 15ºC.

These results indicate that the incorporation of Mg into the carbonate tests of *A. tepida* and *H. depressa* is affected in different ways depending on the Ω component that varies. This implies that Ω does not directly control the changes observed in the Mg incorporation for both species. Similarly, the incorporation of Sr into the test of *A. tepida* might not be directly affected by Ω (Figure 6B).

4.3.5. Effect of CO_3^{2-} and Ca^{2+} concentrations on D_{Mg} and D_{Sr}

As discussed in section 3.4, the $[CO_3^{2-}]_{sw}$ and the $[Ca^{2+}]_{sw}$ have contrasting impacts on Mg incorporation for both benthic species (Figure 6A and C). Moreover, the effect of $[CO_3^{2-}]_{sw}$ on Mg incorporation into the test of *A. tepida* is not significant ($p > 0.05$), in contrast to the impact of changing $[Ca^{2+}]_{sw}$ ($p < 0.05$) (present study and Raitzsch et al., 2009; Figure 6A and C). This implies that within the studied range $[Ca^{2+}]_{sw}$ somehow controls Mg incorporation into carbonate tests of *A. tepida*, overriding possible effects of $[CO_3^{2-}]_{sw}$. Although the $[CO_3^{2-}]_{sw}$ also had a less pronounced effect on D_{Mg} of *H. depressa* than $[Ca^{2+}]_{sw}$ (Figure 6C), the experiments set by Raitzsch et al. (2009) were affected by differences in the Mg/Ca ratios of the seawater solutions used. Therefore, it is not possible to entirely deconvolve the effect of the $[Ca^{2+}]_{sw}$ on Mg incorporation for *H. depressa*.

We observe a significant increase in D_{Sr} of *A. tepida* within the $[CO_3^{2-}]_{sw}$ interval between 158 and 432 µmol/kg (Ω between 4 and 10) ($p < 0.05$; Figure 6B), while higher $[Ca^{2+}]$ does not significantly affect the D_{Sr} (Raitzsch et al., 2009; Figure 6B). The effect of $[CO_3^{2-}]_{sw}$ on D_{Sr} of *H. depressa* is not significant ($p > 0.05$) while the $[Ca^{2+}]_{sw}$ has a significant effect on D_{Sr} (Figure 6D) (Raitzsch et al., 2009). Both studies show an increase in D_{Sr} of *H. depressa* with Ω, indicating that this parameter correlates with Sr incorporation only when $[Ca^{2+}]_{sw}$ varies. According to Raitzsch et al. (2009), part of the increase in D_{Sr} observed at higher $[Ca^{2+}]_{sw}$ might be related to the difference in Mg/Ca ratios of the seawater between the experimental solutions. This might be responsible for changes in Mg incorporation into the test which distorts the mineral lattice (Mucci and Morse, 1983) and allow more Sr ions to be incorporated. Therefore, the response of D_{Sr} to variable $[Ca^{2+}]_{sw}$ is likely to be partly influenced by this factor besides the effect of $[Ca^{2+}]_{sw}$.

4.3.6. Biomineralization and the carbonate chemistry of surrounding seawater

Based on the contrasting results on Mg and Sr incorporation obtained for [Ca^{2+}]$_{sw}$ versus [CO$_3^{2-}$]$_{sw}$ manipulations, we suggest that two independent mechanisms exist that form internal calcification reservoirs. A calcium-concentrating mechanism, which increases the internal [Ca^{2+}], and a mechanism in which bicarbonate from the seawater is transported to a DIC-reservoir, likely to be converted afterwards to increase internal [CO$_3^{2-}$]. The Ca-transporting mechanism may well involve Ca- pumps and/or channels (Erez, 2003; Bentov and Erez, 2006) that essentially transport ions from vacuolized seawater into the internal Ca-pool (de Nooijer et al., 2009b). The other mechanism is responsible for a (transmembrane) transport of bicarbonate (and possibly carbonate) out of vacuolized seawater into an intracellular DIC-pool (Ter Kuile and Erez, 1989; 1991). The independency of the two mechanisms follows from the fact that the availability of carbonate ions does not affect the Mg and Sr incorporation (this study), while altering the saturation state over the same range by varying Ca (Raitzsch et al., 2009) does influence the incorporation of these minor elements. This implies that foraminiferal Mg and Sr incorporation is not directly affected by the saturation state of the medium with respect to CaCO$_3$, but rather Ca^{2+} and (bi)carbonate ions independently: this is summarized in Figure 7.

It is suggested that the Mg-content of the calcite precipitated by foraminifera is a direct result of the specificity of the mechanisms that concentrate Ca^{2+} (Bentov and Erez, 2006; de Nooijer et al., 2009b). This implies that low Mg-species (e.g. *A. tepida*) possess a mechanism that discriminates Ca^{2+} from Mg^{2+} (Figure 7A).

Species which build tests of high-Mg calcite (e.g. *H. depressa* and milliolids) are likely to have a Ca-concentrating mechanism that transports Mg and Ca ions in approximately equal amounts from seawater into the Ca-pool since their Mg/Ca ratio approximates that of calcium carbonates precipitated inorganically from seawater (Mucci and Morse, 1983; Erez, 2003; Segev and Erez, 2006; Raitzsch et al., 2009) (Figure 7B). Selectivity against Mg-ions during transmembrane transport in low-Mg species would also explain the depleted Mg-isotopes found in their calcite (Pogge von Strandmann, 2008) (Figure 7A). This means that any Ca-transport mechanism that allows only some of the Mg to pass would fractionate against heavy isotopes, leaving the internal Ca-pool depleted with respect to Mg-isotopes (Figure 7A). The suggestion that Mg ions are selectively pumped out of the foraminiferal Ca-reservoir would thus result
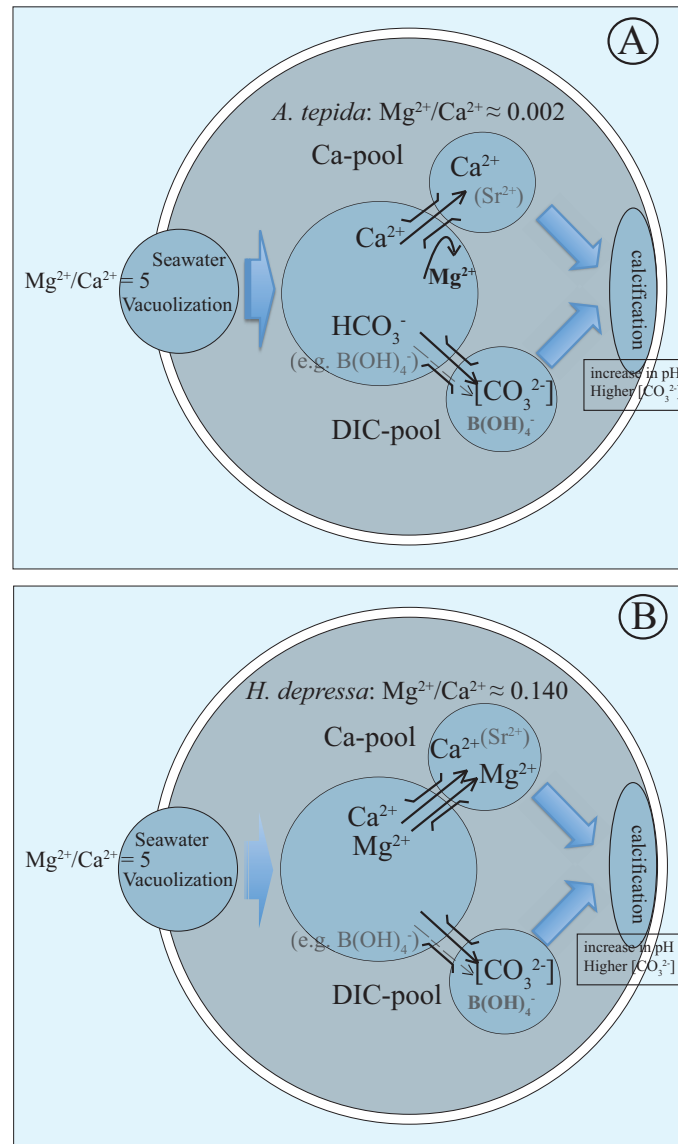
Figure 7. Simplified calcification scheme for A. *Ammonia tepida* and B. *Heterostegina depressa*. For both species, calcification starts with the uptake of seawater in vacuoles. From these vacuoles, both species concentrate Ca and inorganic carbon by transport into internal pools. The specificity of the Ca-transporting mechanism differs greatly between species, resulting in an internal Ca-reservoir with a relatively high Mg/Ca ratio in *H. depressa* (A) and a low Mg/Ca ratio in *A. tepida* (B). The second mechanism, concentrating inorganic carbon from the seawater vacuole, is hypothesized to be responsible for the presence of borate in the inorganic carbon-pool and therefore of B-incorporation into foraminiferal calcite.

in an internal Ca-reservoir enriched with respect to Mg-isotopes. Since this is not the case for a number of species (Pogge von Strandmann, 2008), Mg/Ca ratios in foraminifera cannot be the result of Mg-pumping.

The Mg-isotopic composition of high-Mg species is similar to the result of fractionation during precipitation, resulting in only a small isotopic offset (Kısakürek, pers. comm.) (Figure 7B). The mechanism responsible for the production of the internal inorganic carbon reservoir apparently does not transport (divalent) cations (i.e. Ca^{2+}, Mg^{2+}, Sr^{2+}), remark that is based on the non significant changes observed in Mg and Sr incorporation when [CO$_3^{2-}$] is changed. In fact, such channels or pumps may well exchange the transported negatively charged ions with other negative ions to keep electro-neutrality (Figure 7). Another reason to keep transport of Ca and (bi)carbonate separated, is to prevent spontaneous intracellular nucleation and growth of calcite or aragonite crystals. It may also be that the transmembrane transport of HCO$_3^-$ allows (a relatively small amount of) ions with the same charge and a similar size to enter the inorganic carbon pool. In this respect, some of the borate present in seawater, B(OH)$_4^-$, may well be transported by the (bi)carbonate transporter. This would explain why the δ^{11}B of foraminifera reflect seawater pH: when only borate and not boric acid is transported into the inorganic carbon pool, the isotopic composition of B present in this pool should always reflect the pH of the vacuolized seawater, irrespective of the pH at the site of calcification (Zeebe and Sanyal, 2002; Erez, 2003; de Nooijer et al., 2009a) (Figure 7).

The proposed ion pathways here do not encompass the complete calcification pathway: for example, the molecular transporters are not identified for the species under consideration. It remains therefore unknown in exchange for which ions, or in combination with which ions, Ca^{2+} and HCO$_3^-$ are transported. It could also be that element-specific organic compounds are involved in order to aid ionic transport. Controls during calcification are also omitted here since they are covered in other publications (Erez, 2003; Bentov and Erez, 2006; de Nooijer et al., 2009a; 2009b).

4.4. Conclusions

An increase in [CO$_3^{2-}$] of seawater and thereby Ω slightly promotes the incorporation of Mg and Sr into the carbonate tests of the benthic species *A. tepida* while no significant changes in these two minor elements are observed in the tests of *H. depressa*. Combining these results with experiments in which Ω was altered by changing the seawater Ca

concentration, $[Ca^{2+}]$, indicates that incorporation of divalent cations are affected in different ways depending on the seawater chemical parameter that varies. We suggest that two independent mechanisms are involved in the formation of internal calcification reservoirs. A calcium-concentrating mechanism, which increases internal $[Ca^{2+}]$, and a mechanism in which bicarbonate from the seawater is transported to a DIC-reservoir, likely to be converted afterwards to increase internal $[CO_3^{2-}]$. The independency of the two mechanisms follows from the fact that the carbonate ions do not affect the Mg and Sr incorporation (this study), while altering the saturation state over the same range by varying $[Ca^{2+}]$ (Raitzsch et al., 2009) does influence the incorporation of these minor elements. This implies that foraminiferal Mg and Sr incorporation is not directly affected by the saturation state of the medium with respect to $CaCO_3$, but rather Ca- and (bi)carbonate ions independently.

Acknowledgements

Chapter 5

Inter-individual variability and ontogenetic effects on Mg and Sr incorporation in the planktonic foraminifer *Globigerinoides sacculifer*

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Abstract

In order to investigate the inter-individual and ontogenetic effects on Mg and Sr incorporation, magnesium/calcium (Mg/Ca), and strontium/calcium (Sr/Ca) ratios of the planktonic foraminifer *Globigerinoides sacculifer*, have been measured. Specimens 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 inter-individual variability observed in the Mg/Ca values contributes 2 to 3°C to the apparent temperature variance. Inter-individual variability in Sr/Ca ratios is much smaller than that observed in Mg/Ca values. The variability due to ontogeny corresponds to -0.40 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: $Mg/Ca = (0.55-0.0002*MSD)e^{0.089*T}$ and $Mg/Ca = (0.55-0.0001*MSD)e^{0.089*T}$, respectively, where MSD corresponds to the maximum shell diameter of the individual.

5.1. 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; McConnell and Thunell, 2005; McKenna and Prell, 2004). 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). Therefore, the composition of the foraminiferal carbonate tests can be used as proxy of physical and chemical parameters of the 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 (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 inter-individual and ontogenetic effects on planktonic foraminiferal Mg and Sr incorporation under otherwise controlled physical and chemical seawater conditions. Quantifying the magnitude of these effects is of great importance since, for instance, the use of inter-individual variability to reconstruct seasonality is starting to increase (Sadekov et al., 2008). We have specifically chosen *Globigerinoides sacculifer* (Brady, 1877) as it is a key, mixed layer dwelling, species that is widely used for sea surface temperature reconstructions.

5.2. Methodology

5.2.1. Collection and culturing of foraminifera

Planktonic, symbiont-bearing foraminifera *G. sacculifer* (Figures 1A, B, and 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.
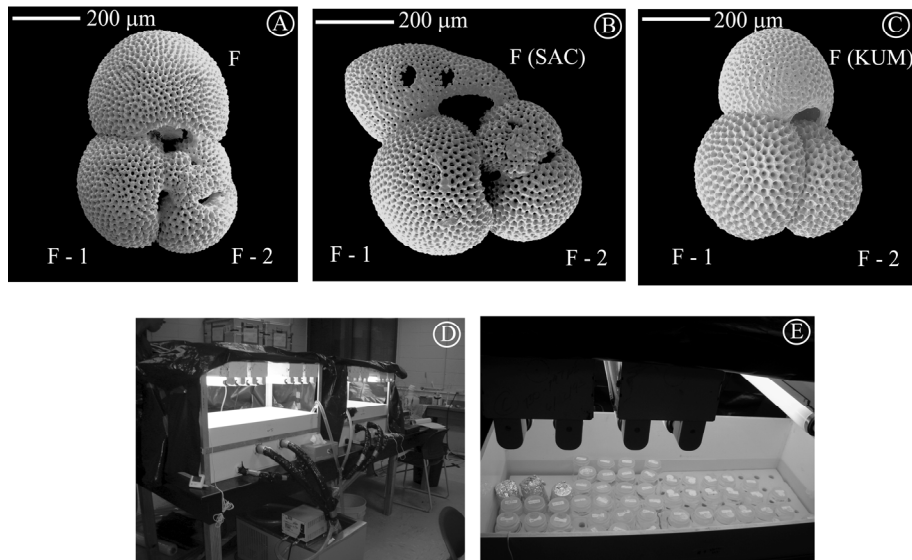
Figure 1. *Globigerinoides sacculifer* analysed by Laser Ablation-ICP-MS and its three different morphotypes based on the size and shape of the last chamber. A. Normal (NOR), B. Sac-like (SAC), and C. Kummerform (KUM). F = ultimate chamber or chamber-stage 19 (in few cases chamber-stage 20); F-1 = penultimate chamber or chamber-stage 18; F-2 = third chamber/ chamber-stage 17 (older than F and F-1). D. Thermostated water baths. E. Inside of water bath containing several pyrex bottles filled with seawater where foraminifera were grown.

We identified and measured individual foraminifera using an inverted light microscope and transferred the specimens to 120-ml Pyrex bottles filled with seawater at the *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 335 µE/m^2/s (12-hr light/12-hr dark cycle) (Figure 1D and E). 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 to 3 additional chambers during the incubations. In order to increase the number of data points for this study, Mg/Ca and Sr/Ca ratios from specimens that were grown under different temperatures and salinities were normalized to values at 26°C and salinity of 36. This was achieved by using existing Mg/Ca and Sr/Ca–temperature and salinity calibrations (Nürnberg et al., 1996b; Kısakürek et al., 2008; Dueñas-Bohórquez et al., 2009).

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) (Figure 1A, B, and 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.

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

Values are given with ± standard deviations
[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 CO$_2$sys (Lewis and Wallace, 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.

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5.2.2. Carbonate chemistry of the culture solutions

Carbonate parameters of the seawater used in the culture experiment were calculated using the CO_2sys program (version 01.05; Lewis and Wallace, 1998). Measurements of salinity, total alkalinity, and pH (NBS scale) of the solutions were performed 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 CO_2sys program were not significantly different (results are presented in Appendix, Table C1).

5.2.3. Sample preparation and analysis

Specimens that grew new chambers were cleaned in a sodium hypochlorite bath (NaClO 5%) for 20 minutes 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 the trace elemental concentrations of individual chambers from single specimens several times.

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. 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 U.S. National Institute of Standards and Technology (NIST) SRM 610 glass with ^{44}Ca as an internal standard. Using calcium as an internal standard is ideal as this element is present at a constant concentration of 40%. 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
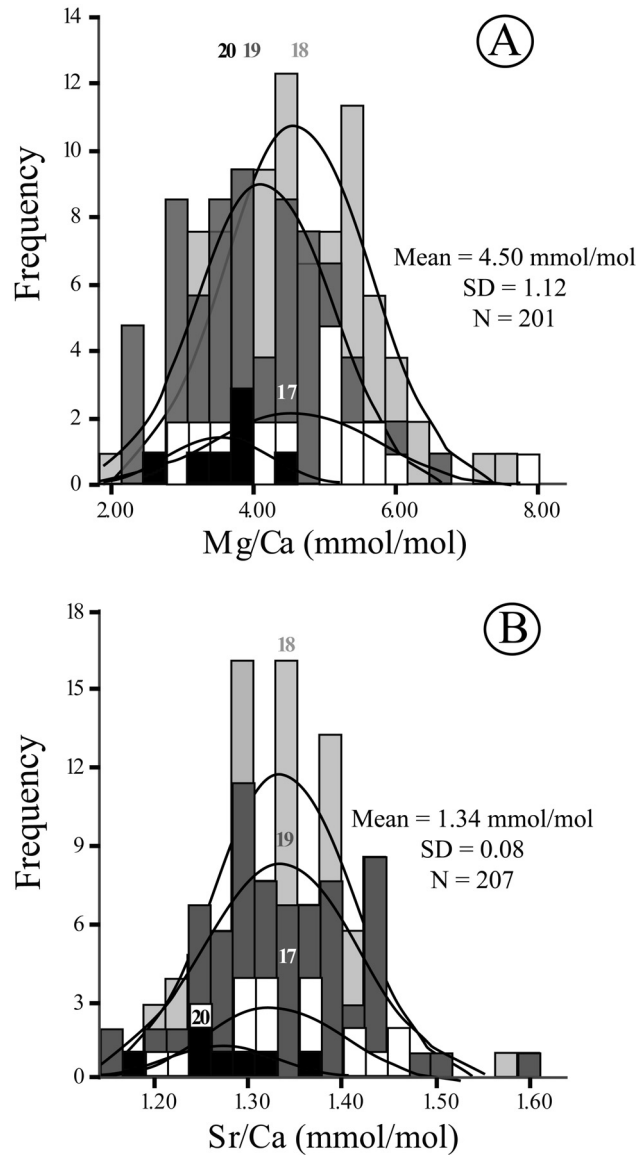
Figure 2. A. Histogram of Mg/Ca and B. Sr/Ca values of four different ontogenetic stages of *Globigerinoides sacculifer*: chamber-stage 17 (white), 18 (light grey), 19 (dark grey), and 20 (black). A frequency distribution for every ontogenetic stage is shown. All chambers were grown at a constant temperature of 26ºC and 36 salinity units.

was included. This standard is an uncommonly homogeneous calcite crystal (Icelandspar) that was analysed by LA-ICP-MS using four different ablation energy densities (results in Appendix, Table C2).

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/Ca and Sr/Ca values are statistically identical (results in Appendix, Table C3). Based on repetitive analyses of the calcite standard throughout the analytical period, relative precision of the LA-ICP-MS analyses for Mg and Sr was around 3% and 3.6%, 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 computer program, which we 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.

5.3. Results

5.3.1. Inter-individual 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 Figures 2 and 3. Each chamber analysed is characterized by a large range of individual Mg/Ca values, for example, from 3 to 8.4 mmol/mol in the case of chamber 17 (Figures 2A and 3A).
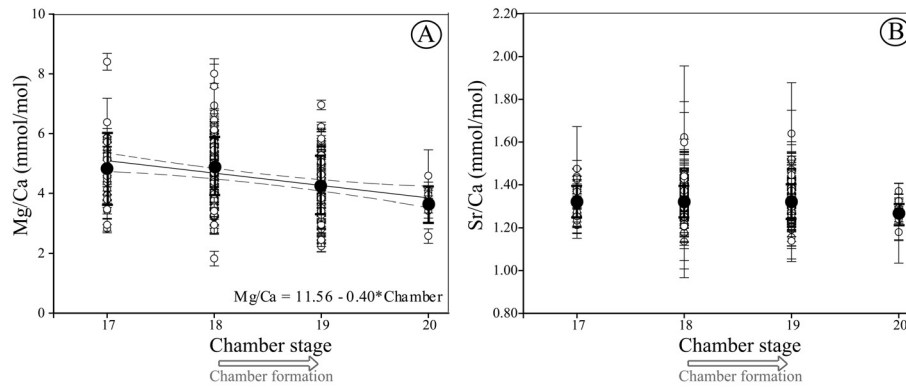
Figure 3. A. Mg/Ca and B. Sr/Ca ratios vs. ontogenetic stage (chamber stage) of *Globigerinoides sacculifer*. All chambers were grown at a constant temperature of 26ºC and 36 salinity units. Error bars indicate ±1standard deviation. The 95% confidence limits of the curve fit are shown by dashed lines.

The variability of Mg/Ca ratios measured per chamber stage is relatively large and range from 0.63 to 1.28 mmol/mol (Table 2). Sr/Ca ratios show a narrower range of values per chamber-stage than Mg/Ca ratios (Figures 2B and 3B) and also a much smaller variability in the Sr/Ca ratio means between chambers. However, some inter-individual variability is still observed (Table 2; Figure 3B).

5.3.2. 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) (Figure 1). The most frequently found Mg/Ca ratios in chambers 18, 19, and 20 are slightly different from each other (Figure 2A), chamber 18 having the highest Mg/Ca value, followed by chambers 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 (Figure 2A). In addition, the Mg/Ca values of *G. sacculifer* slowly decrease from the oldest (chamber 17) to the last chamber (newest) grown in culture (chamber 20) (Table 2; Figure 3A) despite the relatively large inter-individual variability (Figure 3). In contrast, the Sr/Ca ratios are very similar for the four chambers analysed (Table 2; Figures 2B and 3B).

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

Chamber Type	n[a]	Mg/Ca (mmol/mol)	n[a]	Sr/Ca (mmol/mol)
17	23	4.75 ± 1.28	23	1.34 ± 0.08
18	94	4.75 ± 1.08	100	1.34 ± 0.08
19	77	4.18 ± 1.06	77	1.34 ± 0.09
20	7	3.75 ± 0.63	7	1.28 ± 0.06

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

5.4. 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).

5.4.1. Inter-individual variability in Mg/Ca ratios

5.4.1.1. Possible causes of inter-individual 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 (Figures 2 and 3). Standard deviations shown in Table 2, caused by inter-individual variation, are larger than standard deviations which are caused only by analytical plus intrachamber variabilities.

These results demonstrate that neither physical and/or chemical parameters of the seawater, such as daily changes in the temperature and/or salinity of the seawater, are responsible for the variation found in Mg/Ca ratios between individual: all specimens analysed in this study were grown under constant and controlled laboratory conditions. Post depositional dissolution of the carbonate test is also discarded as a source of inter-individual variability.

High inter-individual variability has also been observed in core-top and plankton samples of *Globigerinoides ruber* (Sadekov et al., 2008) in a magnitude similar to the one we report here (0.6 to 1.1 mmol/mol; present study 0.6 to 1.3mmol/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 inter-individual variation.

The high inter-individual variability observed in *G. sacculifer* might alternatively be caused by differences in the amount of symbionts per 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 clear effect on Mg incorporation in *G. sacculifer* (Dueñas-Bohórquez et al., 2009).

Cryptic speciation, which refers to individuals that belong to genetically different species but are morphologically identical, can be discarded as a source of inter-individual 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.

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

		n	F value	p	Experimentally determined responses (in mmol/mol) per chamber stage
Regression	Mg/Ca = 11.56 – 0.40*Chamber	201	13.14	<0.001	-0.40
ANOVA	Between chambers	201	5.36	0.001	
Hochberg *post hoc* Test	Chamber 18 vs. Chamber 19	171	-	0.005	
Regression	Temperature = 29.30 – 0.98*Chamber	201	12.75	<0.001	-0.98

[a]Temperature and salinity were kept constant at 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.

5.4.1.2. Implications of Mg/Ca inter-individual variability for the reconstruction of seasonality

Sadekov et al. (2008) suggested that the inter-individual variability found in Mg/Ca ratios of final chambers of *G. ruber* from core top and plankton tow samples contributes from ~1.6 ± 0.3°C to ~2.5 ± 0.3°C. This value already excludes the inter-individual variation due to seasonality in SST. The inter-individual variability found in *G. sacculifer* contributes 2.5 ± 0.5°C to the apparent temperature variance (standard deviations in Table 4; Figure 5). This implies that the reconstruction of seasonality, based on analysis of single specimen, has an inherent inaccuracy of about 2°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 inter-individual variability might be similar among planktonic foraminiferal species.

5.4.2. Inter-individual variability in Sr/Ca ratios

The inter-individual variability observed in Sr/Ca ratios of *G. sacculifer* is much smaller than the variability observed in Mg/Ca ratios (Table 2; Figure 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 (Figure 3B).

5.4.3. 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). From this Mg/Ca-chamber stage relationship, a decrease of 0.40 mmol/mol of Mg/Ca per chamber stage (from the oldest, chamber 17, to the newest, chamber 20) is estimated (Table 3; Figure 3A). This result is in agreement with studies by Sadekov et al. (2005) on the same species where older chambers (F-1, F-2, 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%).
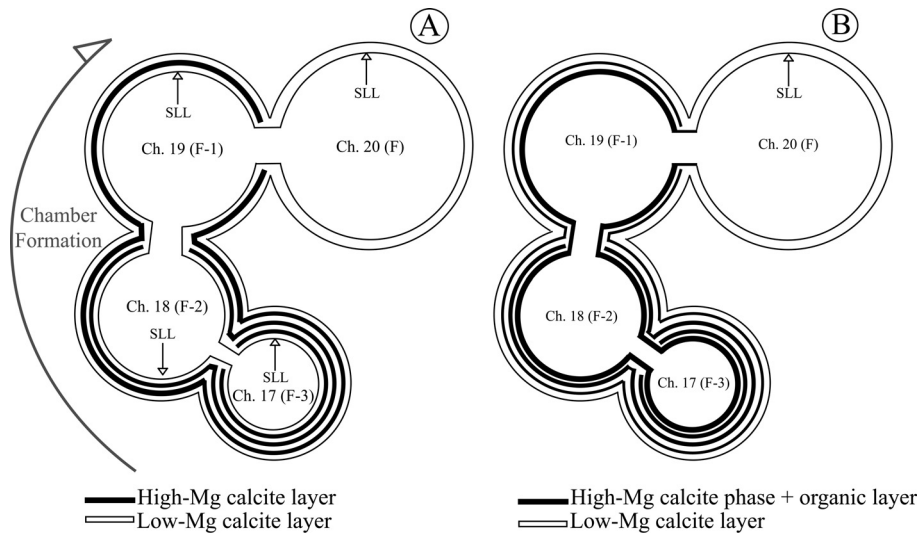
Figure 4. Possible causes of ontogenetic effect on Mg incorporation: A. Presence of single low-Mg layer (SLL) in last chambers (Sadekov et al., 2005). B. Presence of single low-Mg layer (SLL) only in final chamber due to gametogenesis. Double calcite layer formed by a low-Mg calcite plus a high-Mg calcite associated to an organic layer (Bentov and Erez, 2006). Scheme shows differential change in the thickness of the high-Mg and low-Mg double bands with further distance from the newest (last) chamber.

Specimens analysed by Sadekov et al. (2005) were taken from core-top samples. Therefore, this high Mg percentage in older ontogenetic 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* (F-3, 197) = 5.36, $p < 0.05$; where values between brackets correspond to the degrees of freedom for the ontogenetic effect) (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 ($p < 0.05$) (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 (Figures 2A and 3A). This is probably due to the low number of measurements available for these two chamber stages. The high variability observed in Mg/Ca ratios of chamber stage 17 might reflect a transition from natural to culture conditions.

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. We discuss here two hypotheses in order to

explain the observed ontogenetic effect on *G. sacculifer*: 1. daily changes in the pH ($[CO_3^{2-}]$) of the microenvironment of calcification; and 2. presence of organic layers within the carbonate test walls.

Table 4. Temperatures of the chamber stages analysed from *Globigerinoides sacculifer*.

Chamber stage	Mg/Ca (mmol/mol)	T_c (°C)[a]	T_e (°C)[b]	Independent t-test (p)[c]
17	4.75 ± 1.28	28 ± 2.97	26	0.01
18	4.75 ± 1.08	28 ± 2.70	26	0.04
19	4.18 ± 1.06	27 ± 2.94	26	-
20	3.75 ± 0.63	25 ± 1.14	26	-

Values are given with ± standard deviations.
[a]T_c corresponds to calculated temperatures using Nürnberg et al. (1996b) equation: Mg/Ca = 0.39 $e^{0.089*T}$.
[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).

5.4.3.1. Daily changes in the pH ($[CO_3^{2-}]$) of the microenvironment of calcification

According to Sadekov et al. (2005), *G. sacculifer* displays patterns of Mg/Ca banding within its test walls that contain continuous to semi-continuous, narrow high Mg/Ca bands intercalated between thicker but low Mg/Ca bands. 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 but low-Mg calcite bands might be explained by the activity of symbionts (in the case of *G. sacculifer*, the dinoflagellate *Gymnodinium beii*). 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., 1999a). 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 test 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. Based on these results, we cannot be certain that daily changes in the pH of the calcifying microenvironment are fully responsible for the Mg/Ca banding observed within the carbonate test of this species.

Sadekov et al. (2005) also showed that the high-Mg/Ca bands are observed in all but the last chamber. 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 (Figure 4A). 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 (Figure 3A).

The lack of high-Mg bands in the calcite wall of the last chamber (Sadekov et al., 2005) does not agree with the mechanisms of chamber formation described by Erez (2003). This process includes the formation of an organic template and the precipitation of $CaCO_3$ on both sides of this organic layer. Organic layers have great affinity for Mg (Bentov and Erez, 2006); therefore, a band of high-Mg should be present in every newly formed chamber.

We consider that the lack of high-Mg bands in the last chamber shown in Sadekov et al. (2005) can be explained in terms of gametogenesis, where a different calcification mechanism is responsible only for the formation of this chamber (Hamilton et al., 2008). Assuming that every newly formed chamber consists of a high-Mg/low-Mg double layer, we would not expect changes in the high-Mg/low-Mg band ratio nor in the Mg/Ca values between chambers. Therefore, changes in Mg/Ca ratios due to ontogeny, as observed in the present study, could only be explained if we assume that the thickness of the high-Mg and low-Mg double bands changes disproportionately with further distance (chamber stage 19, 18, 17, etc.) from the new (last) chamber (ontogenetic stage 20) (Figure 4B).
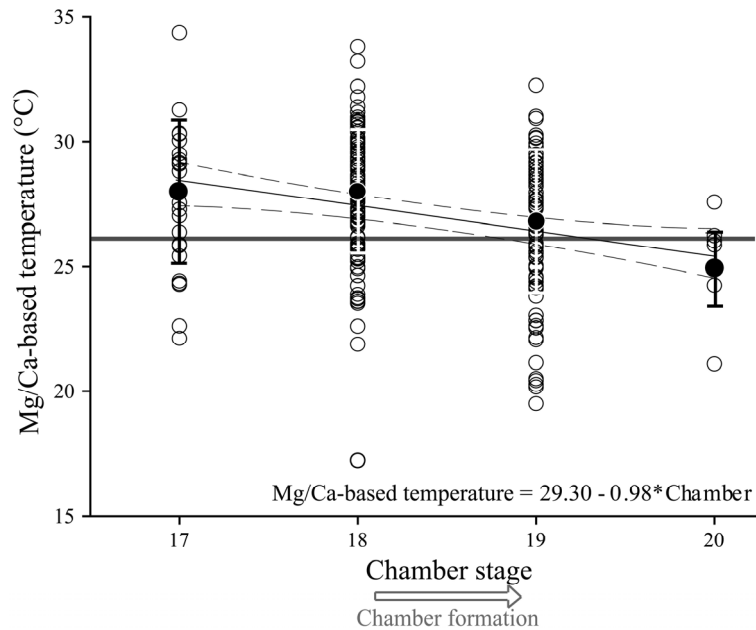
Figure 5. Temperature values based on measured Mg/Ca ratios from each chamber stage of *Globigerinoides sacculifer*. Temperatures were calculated using the following equation: Mg/Ca = 0.39 e0.089*T (Nürnberg et al., 1996). Black line corresponds to the experimental temperature (26ºC). The 95% confidence limits of the curve fit are shown by dashed lines.

5.4.3.2. Presence of organic layers within the carbonate test walls

The presence of organic layers within the carbonate test walls might caused the differential Mg/Ca banding in *G. sacculifer* (Bentov and Erez, 2006). These organic layers are able to easily bind to Mg and might be the source of high-Mg within the foraminiferal carbonate test wall. Experiments carried out with the benthic foraminiferal species *Amphistegina lobifera* show that high-Mg calcite phases are associated with layers that are enriched in organic compounds (Erez, 2003). These results show that organic layers can influence the Mg/Ca ratio in calcite and may explain some of the intratest Mg heterogeneity (Bentov and Erez, 2006).

This hypothesis is supported by the thermodynamic stability of foraminiferal calcium carbonate, since it is more effective for the foraminifer to build test walls with layers of low-Mg rather than high-Mg calcite because the latter has a higher solubility in seawater (Mucci and Morse, 1984). Therefore, we suggest that the ontogenetic effect observed

on Mg incorporation into the carbonate test of *G. sacculifer* might be due to disproportional changes between the thickness of the high-Mg organic layer and the low-Mg calcitic band with further distance from the new (last) chamber (Figure 4B).

5.4.4. Ontogenetic variation of Sr/Ca

No clear ontogenetic effect is observed in Sr/Ca ratios for the last four chamber stages of *G. sacculifer* (Figures 2B and 3B). Our results also show a much smaller inter-individual variability for Sr/Ca ratios than for Mg/Ca ratios. Moreover, Mg/Ca and Sr/Ca values from every chamber stage do not correlate ($p > 0.05$). This suggests that the mechanism responsible for the Mg differences between ontogenetic stages (chambers) does not influence Sr incorporation. In inorganic and biogenic calcite, the Sr/Ca ratio is known to increase with growth rate (Lorens, 1981; Nehrke et al., 2007; Tang et al., 2008; Kısakürek et al., 2008). The lack of a clear trend in Sr/Ca ratios of *G. sacculifer* suggests that growth rate was relatively constant during the last four ontogenetic stages.

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

Two mechanisms have been proposed to explain the differential element-banding in the carbonate test walls of *G. sacculifer*: 1. daily changes in the pH of the calcifying microenvironment due to symbiotic activity (photosynthesis) and respiration (Eggins et al., 2004; Sadekov et al., 2005); 2. presence of Mg-enriched organic layers within the carbonate test walls (Bentov and Erez, 2006). If the first mechanism is responsible for the differential Mg banding, we would also expect Sr banding patterns within the carbonate test. Previous culture studies of different species of planktonic foraminifera (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 change of 200 to 400 μmol/kg for the daily $[CO_3^{2-}]$ variation in the calcifying microenvironment (photosynthesis vs. respiration) (Wolf-Gladrow et al., 1999a), this variation 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. This difference between high-Sr and low-Sr bands within the test wall of *G. sacculifer* was not observed by Eggins et

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al. (2003). Moreover, we do not observe significant differences in Sr/Ca ratios between chamber stages (Figures 2B and 3B).
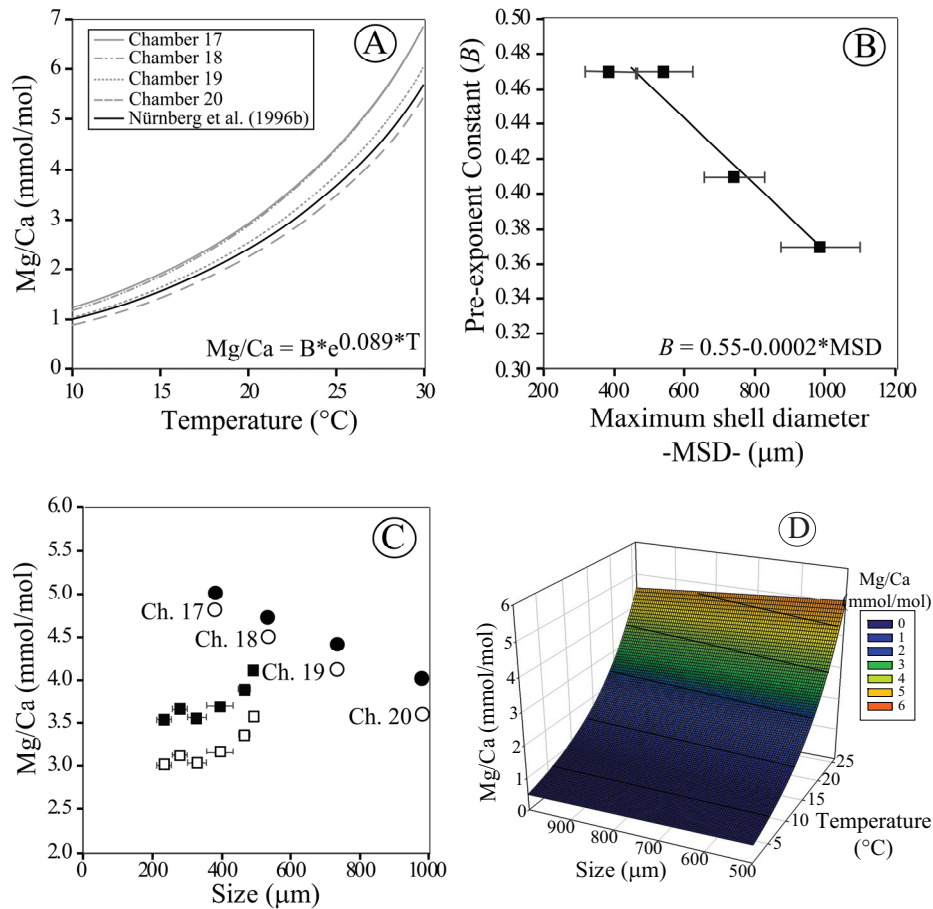
Figure 6. A. Mg/Ca–temperature calibrations calculated from the equation Mg/Ca=B*e0.089T for the last four chamber stages of *Globigerinoides sacculifer*; B. Dependence of the pre-exponent constant *B* of every chamber stage on average maximum shell diameter –MSD- (Hemleben and Bijma, 1994); C. Calculated whole test Mg/Ca ratios based on single chamber analyses. Open circles correspond to Mg/Ca values per chamber, closed circles correspond to Mg/Ca for the whole foraminiferal test. 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 ºC), using the Mg/Ca-temperature calibration of Nürnberg et al. (1996b); D. Estimated Mg/Ca ratios for whole foraminiferal test vs. size and temperature. Black lines indicate constant temperature.

Therefore, there is strong evidence to reject the hypothesis of a daily pH change in the microenvironment of calcification as the control mechanism of trace element banding within the test walls.

Based on our Mg/Ca and Sr/Ca results, the presence and variable thickness of the high-Mg organic/low-Mg calcitic double band is the most suitable mechanism that can explain the ontogenetic effect on Mg incorporation.

Kunioka et al. (2006) show the presence of co-occurring high-Mg/Ca and Sr/Ca banding within test walls of planktonic foraminifera *Pulleniatina obliquiloculata* which can be matched to organic-rich layers. The high-Sr bands can be enriched by up to 21% Sr in comparison with low-Sr bands. This suggests that the distribution of Mg/Ca and Sr/Ca within the carbonate test wall might be species-specific. However, those observations are based on the analysis of one specimen and should therefore be considered with caution.

Table 5. Estimated Mg/Ca ratios for every chamber stage and whole *Globigerinoides sacculifer* specimens.

Chamber stage	Shell size (µm)	Shell weight (µg)	Mg/Ca per chamber (mmol/mol)[a]	Mg/Ca for whole specimens (mmol/mol)[b]
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]Calculated using Equation 1: $Mg/Ca = (0.55-0.0002*MSD)e^{0.089*T}$ at a constant temperature of 26ºC.
[b]Calculated using Equation 2: $\Sigma_{Ch0-20} (Mg/Ca_{Ch}* W_{Ch}) = Mg/Ca_T * W_T$.

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

Calcification temperatures based on measured Mg/Ca ratios of individual chamber, have been calculated using the only available Mg/Ca-temperature calibration from laboratory cultures (Nürnberg et al., 1996b; Figure 5). 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; Figure 5).

The Mg/Ca-based temperatures for chamber stages 17 and 18 are significantly higher than the temperature predicted by the Mg/Ca-temperature calibration of Nürnberg et al. (1996b) ($p < 0.05$; Table 4). On the other hand, no significant difference is observed between the Mg/Ca ratio obtained by using the calibration of Nürnberg et al. (1996b) and the Mg/Ca values of the last 2 chamber stage (19 and 20). 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. 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 = $0.39\ e^{0.089*T}$ in Nürnberg et al., 1996b) but with varying pre-exponent constants (Figure 6A). This equation is used as it is based on data from laboratory culture experiments of *G. sacculifer*, which means this data set is similar to the results presented here. 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 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*) vs. MSD, we obtain a linear correlation (Figure 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:

$$Mg/Ca_{chamber} = (0.55-0.0002*MSD)e^{0.089*T} \quad \text{(Equation 1)}$$

This formula is based on the observed change in $Mg/Ca_{chamber}$ ratios in the last four chamber stages (17 to 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 Equation 1 (Table 5; Figure 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:

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

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 Equation 2 well beyond the calibration interval (last four ontogenetic stages), this is justified by the relative small contribution of the early test carbonate. 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:

$$Mg/Ca = (0.55-0.0001*MSD)e^{0.089*T} \quad \text{(Equation 3)}$$

This formula is based on the estimated change in Mg/Ca ratios according to the size of the whole specimen (Figures 6C and D).

A more gradual decrease in Mg/Ca values is observed with increasing foraminiferal size when considering whole tests (Figure 6C). Elderfield et al. (2002) reported lower Mg/Ca ratios of whole foraminifer test than the Mg/Ca values measured here (Figure 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 (26ºC). 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 (Figure 6C) where physical and chemical parameters of the seawater were kept constant. Therefore, the Mg/Ca-size relationship

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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*). Following the same procedure, we calculated the $[CO_3^{2-}]$ based on these Holocene Mg/Ca ratios using the formula proposed by Russell et al. (2004). The $[CO_3^{2-}]$ values obtained (between 382 and 410 µmol/kg) are relatively high for surface seawater. This indicates that none of these parameters can explain the increase in Mg/Ca with size. Therefore, other parameters, 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 should be taken into account as a possible source of variation in Mg/Ca ratios with size.

Recently, Hamilton et al. (2008) showed that the planktonic foraminifer *O. 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. Without the addition of lower-Mg GAM-calcite, the ontogenetic trend observed in the cultures 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.

5.5. Conclusions

The inter-individual Mg/Ca variability found in the planktonic foraminifer *G. sacculifer* is an important factor that needs to be taken into account. This inter-individual variability contributes 2 ± 1°C to the apparent temperature variance. Inter-individual 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 ratios decrease by 0.40 mmol/mol per ontogenetic stage (from the oldest to the newest chamber). This is valid for 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). This Mg banding is caused by the presence of narrow high-Mg calcite phases associated with organic layers intercalated between thicker but low Mg/Ca-calcite bands (Bentov and Erez, 2006). The Mg/Ca ratio variations due to ontogeny may be explained by a differential change in the thickness of the high-Mg and low-Mg double 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 can confidently 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 equations based on Mg/Ca measurements of the last four ontogenetic (chamber) stages and whole foraminiferal test: $Mg/Ca = (0.55-0.0002*MSD)e^{0.089*T}$ and $Mg/Ca = (0.55-0.0001*MSD)e^{0.089*T}$, respectively, where MSD corresponds to the maximum shell diameter (MSD) of the individual.

Acknowledgements

Chapter 6

Changes in trace and minor element incorporation in the deep benthic foraminifera *Cibicidoides pachyderma* as a function of microhabitat and ontogeny

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Abstract

Specimens of deep-sea benthic foraminifera *Cibicidoides pachyderma* were collected from core-top samples from the Bay of Biscay and the chemical composition of their test calcite was analysed. Overall composition of the tests is in line with earlier studies, even though the Mg concentration is appreciably lower. Inter and intra-individual variations of the minor and trace element composition of the tests reflect changes in chemistry of bottom and pore-water. The Ba and Mn incorporation into the calcite test indicates vertical migration within the top few centimeters of the sediment. The lack of inter and intra-individual variation in the Sr/Ca ratios indicates that Sr is apparently not affected by either pore-water chemistry, migration patterns or changes in vital effects. The intra-individual variability in Mg/Ca ratios cannot be explained by migration alone, whereas changes in Ba and Mn concentrations in the pore-water are more directly reflected in the test carbonate. A possible explanation for the observed changes in foraminiferal Mg/Ca ratios involves the presence of food cysts around specimens during early life stages at the sediment-water interface. The presence of a food cyst influences pH, which is reflected in the foraminiferal Mg incorporation. The Mg/Ca inter-individual variability found in *C. pachyderma* potentially contributes 2°C to the apparent temperature variance.

6.1. Introduction

Carbonate tests of deep-sea benthic foraminifera are frequently used as proxy signal carriers for physical and chemical parameters of the seawater. Paleoceanographic and paleoclimatological studies increasingly rely on proxies based on trace metals incorporated in foraminiferal tests. Depending on their biogeochemical behaviour in seawater, these trace metals can be used as physical proxies (e.g. Mg, Sr), nutrient proxies (e.g. Ba) and diagenetic proxies (e.g. Mn).

Trace elements such as Mg and Sr, which are incorporated into foraminiferal calcitic tests, behave conservatively in the oceans and are often referred to as physical proxies. They occur in nearly constant proportion to calcium in seawater and their variability in the foraminiferal tests is related to physical parameters such as temperature and salinity (Lea, 1999). The Mg incorporated into foraminiferal tests, often expressed as Mg/Ca ratios, primarily varies according to changes in seawater temperature (Rosenthal et al., 2006; Elderfield et al., 2006; Lear et al., 2002; Martin et al., 2002). Even though temperature is the overriding control on foraminiferal Mg/Ca ratios (Rosenthal et al., 1997), other seawater parameters, such as the carbonate concentration ($[CO_3^{2-}]$) and the salinity of the seawater, also affect this trace element (Yu and Elderfield, 2008; Healey et al., 2008; Raitzsch et al., 2008). Thus these parameters can potentially bias Mg/Ca-temperature calibrations. Similarly, the Sr concentration of benthic foraminiferal tests has been used as a temperature proxy in aragonitic foraminifera (Reichart et al., 2003), but in most species reflects the carbonate chemistry of the ambient seawater (Rosenthal et al., 2006; Rathmann and Kuhnert, 2008).

Trace elements present in foraminiferal tests are also used to reconstruct the composition of ambient seawater. These elements are not constant in their ratio to Ca in seawater since they are part influenced by the biogeochemical cycling in the ocean. Therefore, changes in their concentrations in foraminiferal calcitic tests reflect changes in the seawater composition and thus biogeochemical cycles.

Barium (Ba) is a trace element that behaves like a nutrient in the oceans, which means it is removed from solution in surface waters through the precipitation of barite, $BaSO_4$ (Bishop, 1988) and incorporated into sinking organic particles that dissolve in deep seawater and in the sediments. Barium shares these key aspects of its behaviour with silica and alkalinity. Consequently, this trace element can be used as tracer for seawater nutrient distribution and provides relevant information on ocean circulation and alkalinity shifts (Lea and Boyle, 1989). The incorporation of

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Ba into benthic foraminiferal tests mainly depends on bottom seawater Ba concentration, i.e. higher Ba concentrations in seawater cause an increase in foraminiferal Ba/Ca ratios (Lea and Boyle, 1989; McCorkle et al., 1995) found in core-top samples of *Cibicidoides wuellerstorfi* that D_{Ba} decreased with water depth, result that was explained by a possible loss of trace elements due to a dissolution effect.

Manganese (Mn) is a trace element commonly used as diagenetic proxy when studying foraminiferal fossil tests since it can be strongly influenced by post-depositional processes (Lea, 1999). However, the Mn concentration in the tests of living foraminifera reflects the Mn concentration of the ambient seawater (Reichart et al., 2003). Since dissolved Mn in pore water shows large concentration changes in the upper few centimeters, it could potentially be used to determine the microhabitat preferences of foraminifera.

Deep-sea benthic foraminifera belonging to the genus *Cibicidoides* are typical epibenthic organims that inhabit sediment areas that are in contact with well oxygenated bottom water. *Cibicidoides* species are worldwide distributed and occur at a wide range of depths (Jorissen, 1999). Living at or below the sediment-water interface, the trace element composition of their calcitic test is thought to reflect the chemical composition of the bottom water (Lea, 1999). However, this typical epifaunal benthic foraminifer has also been observed in slightly infaunal habitats (e.g. Corliss, 1991; McCorkle et al., 1997; Fontanier et al., 2002), which implies that this species can record changes in the pore water chemistry. The chemistry of the pore water is controlled by geochemical gradients associated with ongoing early diagenesis. This might have large repercussions for the foraminiferal incorporation of elements with a nutrient-like behaviour (e.g. Ba), redox-sensitive elements (e.g. Mn), and possibly elements influenced by changes in the carbonate chemistry of the seawater.

Here, we present a laser ablation ICP-MS study of inter and intra-individual variation of trace elements in core-top samples of living *Cibicidoides pachyderma*, Rose Bengal stained, from the Bay of Biscay, North East Atlantic. The chemical composition of the pore water have been compared to the trace element composition of single chambers in order to determine possible migration patterns.

6.2. Methodology

6.2.1. Collection of foraminifera

Deep-sea benthic foraminifera *C. pachyderma* were collected from a deep station in the Bay of Biscay (Figure 1). Station B is located at 550 m depth (43º 49' 98" N, 2º 23' 04" W) in the Sub-Artic Intermediate Water (SAIW) (van Aken, 2000a). Bottom water has a salinity of 35.60 and a temperature of ~11ºC (Van Aken, 2000a). Specimens of *C. pachyderma* were picked from a core that was collected with a Barnett-type multi-corer (Barnett et al., 1984). The core sediment was sliced every 0.5 cm, and the intervals 0-0.5 cm and 0.5-1 cm were selected for further collection of specimens of *C. pachyderma*. Sediments were stored in 500 cm^3 bottles containing Rose Bengal stain dissolved in ethanol 95% (1 g/l) (Fontanier et al., 2002). Rose Bengal is used to identify living foraminifera since it binds to their protoplasm (Walton, 1952). Only stained specimens from the sediment fraction 150-600 µm were selected for trace element measurements.
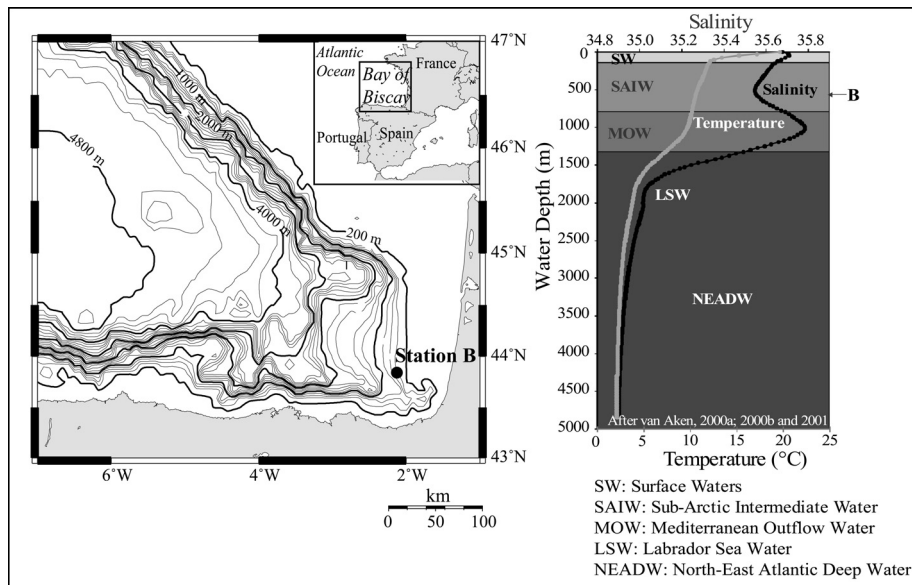
Figure 1. Location of station B in the Bay of Biscay (after Mojtahid).

Results of inter-individual variation presented here are based on analysis of Mg/Ca, Sr/Ca, Ba/Ca, and Mn/Ca ratios of 12 specimens of *C. pachyderma*. Every individual was measured three times in the centre of their umbilical side (Figure 2). The intra-individual variation was determined by analysing individual chambers that were named as follows: F (final last chamber), F-1 (penultimate chamber), F-2 (chamber before that), etc.

6.2.2. Sample preparation and analysis

Foraminifera were ablated using a deep ultraviolet wavelength laser, which is essential for the reproducible ablation of the fragile foraminiferal tests. Set-up and instrument settings resulted in uniform ablation craters, with no signs of fracturing (Figure 2). Samples were ablated using a Lambda Physik excimer laser (193 nm) using a shot repetition rate of 8 Hz and energy density at the sample surface of 2 J/cm^2. UV ablation is critical as the poor absorption of infra-red radiation by carbonates can cause uncontrolled catastrophic damage during ablation (Jackson et al., 1992), which would not only result in unreliable element ratios but also destroy the foraminiferal tests.

Table 1. Pore-water chemical composition at Station B, Bay of Biscay. [a]Data from Anschutz (pers. comm.) which corresponds to samples taken during the Foramprox2 cruise from 2008.

Depth in sediment (cm)	Mn^{2+} (µmol/L)[a]
0	0.1
0.25	0.0
0.75	0.0
1.25	0.0
1.75	0.3
2.50	3.6
3.50	22.3
4.50	12.5
5.50	10.9
7.00	14.1
9.00	17.8
11.00	18.0
13.00	18.4
17.00	18.9
21.00	17.1
25.00	15.7
29.00	20.7

Ablation craters were 80 µm in diameter, with a typical ablation time of up to 60 seconds. The ablated material was transported by a

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continuous He-flow and mixed with an Ar make-up gas before injection into the Ar-plasma of the quadrupole ICP-MS instrument (Micromass Platform). Signal response for each element was carefully monitored with respect to time and depth during ablation. Calibration was performed against NIST SRM 610 glass using the concentration data of Pearce et al. (1997) with Ca as an internal standard. Calcium is ideal, because i) the concentration is constant at 40 wt% in all foraminiferal tests, and ii) direct comparisons can be made with trace metal to Ca ratios from wet-chemical studies. There was no fractionation during ablation between Ca and the other elements of interest, especially at the low depth/diameter ratio of the ablation craters produced in this study (Mank and Mason, 1999).

Three measurements of the standard glass (NIST SRM 610) were made followed by two analysis of the calcite crystal (Icelandspar) before the ablation of 9 foraminiferal samples. Blank corrections were made by measuring background count rates with gas flowing through the ablation chamber before each ablation event.
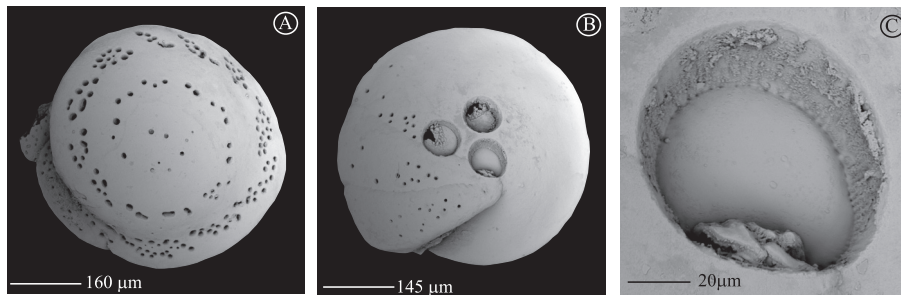
Figure 2. *Cibicidoides pachyderma* analysed by Laser ablation ICP-MS. A. Spiral side. B. Umbilical side. C. Detail of laser-ablation crater.

The concentration of Ba was calculated using ^{138}Ba. Although an isobaric overlap with ^{138}La and ^{138}Ce occurs at ^{138}Ba, this was insignificant when measuring carbonates due to the fact that the more abundant ^{139}La and ^{140}Ce isotopes were below detection limits. This was confirmed by the very good correlation of the Ba contents based on ^{137}Ba and ^{138}Ba (R^2 = 0.95). However, since the detection limit for Ba is higher for the less abundant ^{137}Ba, the ^{138}Ba isotope was used for calculating concentrations. Mg concentrations are calculated using the average of ^{24}Mg and ^{26}Mg. For Ca we used ^{42}Ca, ^{43}Ca, and ^{44}Ca. Although only ^{44}Ca was used for calculating concentrations, the ratios between the different isotopes were used as an additional response check. The mono-isotopic element Mn was quantified

using ^{55}Mn counts, whereas Sr concentrations were based on ^{88}Sr. Contamination with clay particles was monitored by ^{27}Al counts.

Relative analytical errors, based on repeated analyses of an uncommonly homogeneous calcite crystal (Icelandspar) (Chapter 4 in this book) are 4% for Mg, 4% for Sr, and 5% for Mn. Ba concentration in this calcite crystal is too low for our purposes. The differences between sediment intervals analysed were evaluated by comparing the mean concentrations of every foraminiferal trace element measured using the independent t- test. This was done after verifying the data with the basic assumptions of a normal distribution. Non-parametric statistics (Friedman's Test) were used when the data did not follow a normal distribution.

6.3. Results

6.3.1. Trace element distribution

The foraminiferal element composition in terms of Mg/Ca, Sr/Ca, Ba/Ca, and Mn/Ca ratios was plotted against ^{27}Al–counts to ensure no particles adhered to the calcitic test introduced errors in the trace element analyses.

No correlation is observed between the trace elements analysed and ^{27}Al-counts in the middle section of the umbilical side for the 12 specimens of *C. pachyderma* (Figures 3A-D). However, when plotting Mn/Ca and Ba/Ca against ^{27}Al-counts we observe a positive correlation when taken into account some of the measurements (inner squares in Figures 3C and D). Although, this does not necessarily imply that these measurements indicate some sort of contamination, they were discarded from further analyses to avoid any possible bias.

6.3.2. Inter-individual variation of Mg/Ca, Sr/Ca, Ba/Ca, and Mn/Ca

Results of Mg/Ca and Sr/Ca shown in this section correspond to the combined data of specimens of *C. pachyderma* collected from two sediment intervals: 0-0.5 cm (top interval) and 0.5-1 cm (deep interval). This approach was used since the trace element values obtained in these two intervals were not significantly different (p > 0.05). Every individual was measured three times with the exception of three specimens that were measured twice since they were not large enough to do more analysis (Table 2; Figure 4). The Mg/Ca ratios are characterized by values ranging from 0.70 to 1.81 mmol/mol (Figures 4A and B). The variation in

Mg/Ca ratios obtained from the analysis of 12 specimens corresponds to 0.22 mmol/mol, which accounts for approximately 20% of the average Mg/Ca value for this species of benthic foraminifera at this station (Table 2; Figures 4A and B). The Sr/Ca ratios show a narrower range of values than Mg/Ca ratios (1.04 to 1.35 mmol/mol; Figures 4C and D). The inter-individual variability here corresponds to 0.05 mmol/mol, which is equivalent to 4% of the Sr/Ca average value of the sample set (Table 2).
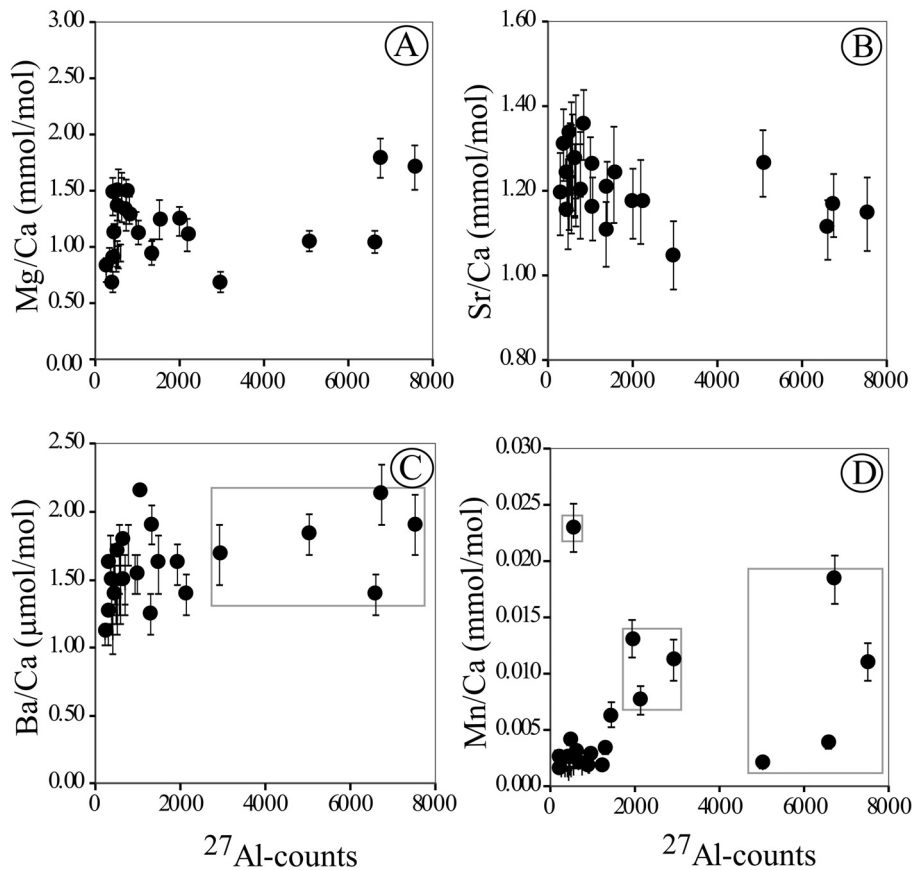
Figure 3. A. Mg/Ca ratios versus 27Al-counts; B. Sr/Ca versus 27Al-counts; C. Ba/Ca versus 27Al-counts; D. Mn/Ca versus 27Al-counts. Inner squares in C and D correspond to measurements that were considered outliers or possibly biased by Al-riched particles. Measurements shown here correspond to all laser-ablation analyses performed in the 12 individuals studied (see Table 2).

Table 2. Trace element composition of specimens of *Cibicidoides pachyderma*.

Chamber	No. specimen	Mg/Ca (mmol/mol) [a]	Sr/Ca (mmol/mol) [a]	Ba/Ca (µm/mol) [a]	Mn/Ca (mmol/mol) [a]
Top Interval (0.0-0.5 cm)	1	0.90 ± 0.10	1.30 ± 0.09	1.09 ± 0.07	0.001 ± 0.0002
	2	0.80 ± 0.11	1.19 ± 0.10	1.09 ± 0.07	0.003 ± 0.0005
		0.95 ± 0.08	1.20 ± 0.07	1.31 ± 0.07	**0.023**[c] ± 0.0022
	3	1.38 ± 0.14	1.32 ± 0.09	1.31 ± 0.15	0.001 ± 0.0004
		0.95 ± 0.10	1.09 ± 0.08	1.24 ± 0.15	0.001 ± 0.0004
		-	1.22 ± 0.09	1.67 ± 0.22	0.001 ± 0.0004
	4	1.23 ± 0.13	1.17 ± 0.08	1.60 ± 0.15	**0.013**[c] ± 0.0016
		1.51 ± 0.16	1.20 ± 0.09	1.67 ± 0.15	0.002 ± 0.0004
		1.10 ± 0.12	1.19 ± 0.09	1.60 ± 0.15	0.001 ± 0.0002
	5	0.91 ± 0.10	1.28 ± 0.10	1.24 ± 0.15	0.001 ± 0.0004
		1.08 ± 0.12	1.14 ± 0.09	1.09 ± 0.15	0.002 ± 0.0004
		0.70 ± 0.09	1.04 ± 0.08	**1.67**[c] ± 0.22	**0.011**[c] ± 0.0018
	6	1.72 ± 0.20	1.14 ± 0.09	**1.89** [c] ± 0.22	**0.011**[c] ± 0.0016
		1.52 ± 0.18	1.25 ± 0.10	1.38 ± 0.15	0.002 ± 0.0004
		1.46 ± 0.17	1.23 ± 0.10	1.24 ± 0.15	0.001 ± 0.0004
	7	0.95 ± 0.12	1.25 ± 0.10	1.38 ± 0.15	0.001 ± 0.0002
		0.70 ± 0.09	1.26 ± 0.11	1.60 ± 0.22	0.002 ± 0.0004
		1.12 ± 0.14	1.17 ± 0.10	1.38 ± 0.15	**0.008**[c] ± 0.0013
	Average	*1.10*	*1.21*	*1.32*	*0.002*
	SD[b]	*0.26*	*0.05*	*0.19*	*0.001*
Deep interval (0.5-1.0 cm)	8	1.29 ± 0.11	1.16 ± 0.07	1.38 ± 0.15	0.004 ± 0.0005
	9	1.27 ± 0.11	1.26 ± 0.08	1.46 ± 0.15	0.002 ± 0.0004
		1.34 ± 0.12	1.35 ± 0.08	1.75 ± 0.15	0.001 ± 0.0004
		1.22 ± 0.11	1.25 ± 0.08	1.53 ± 0.15	0.002 ± 0.0002
	10	1.06 ± 0.10	1.26 ± 0.08	**1.82** [c] ± 0.15	**0.002**[c] ± 0.0004
		0.98 ± 0.09	1.19 ± 0.07	1.89 ± 0.15	0.003 ± 0.0005
		1.05 ± 0.10	1.10 ± 0.07	**1.38** [c] ± 0.15	**0.004**[c] ± 0.0005
	11	1.81 ± 0.18	1.16 ± 0.07	**2.11** [c] ± 0.22	**0.018**[c] ± 0.0022
		1.13 ± 0.11	1.15 ± 0.07	1.53 ± 0.15	0.003 ± 0.0004
		0.87 ± 0.08	1.27 ± 0.08	1.46 ± 0.15	0.002 ± 0.0004
	12	1.25 ± 0.17	1.23 ± 0.11	1.60 ± 0.22	0.006 ± 0.0011
		1.43 ± 0.20	1.30 ± 0.12	1.38 ± 0.22	0.003 ± 0.0007
		1.41 ± 0.20	1.19 ± 0.11	1.46 ± 0.22	0.003 ± 0.0005
	Average	*1.25*	*1.21*	*1.57*	*0.003*
	SD[b]	*0.13*	*0.05*	*0.20*	*0.001*

Values are given with ± standard deviations.
[a]Standard deviation corresponds to analytical error plus intra-chamber variability.
[b]SD= standard deviation that corresponds to inter-individual variation.
[c]Numbers in bold correspond to outliers or suspect data that was not taken into account for further discussion.

Foraminiferal Ba/Ca and Mn/Ca ratios obtained from the two sediment intervals analysed were significantly different ($p < 0.05$), being the foraminiferal trace element values from the deepest sediment interval the highest (Figures 4E-H). The Ba/Ca values for the top sediment interval (0.0-0.5 cm) ranged from 1.09 to 1.60 µmol/mol, while the Ba/Ca range for the deeper interval was 1.38 to 1.89 µmol/mol (Figures 4E and F). The inter-individual variability of Ba/Ca for both intervals is on the same order of magnitude as the analytical uncertainty (Table 2) and corresponds to

0.20 µmol/mol for both sediment intervals. On the other hand, the average Mn/Ca value for the top sediment interval (0.0-0.5 cm) was 0.0017 ± 0.0005 mmol/mol, while the average Mn/Ca for the deeper interval was 0.0032 ± 0.001 mmol/mol (Figures 4G and H). The inter-individual variability of Mn/Ca for both intervals is on the same order of magnitude as the analytical uncertainty (Table 2) and corresponds to 0.001 µmol/mol for both sediment intervals.

6.3.3. Intra-individual variation of Mg/Ca, Sr/Ca, Ba/Ca, and Mn/Ca

The differences in Mg/Ca ratios measured on individual ablation craters within individuals are in most cases not significant (Figure 4B). Similarly, Sr/Ca values within individuals do not show a significant variability (Figure 4D). In contrast, Ba/Ca and Mn/Ca ratios show within test variability of the same order of magnitude as between tests, albeit that this variability is close to the analytical uncertainty (Figures 4F and H).

   The trace element composition of individual chambers was analysed in three selected specimens of *C. pachyderma* (Figure 6), two of which were collected from the top interval (0.0-0.5cm) (S1 and S2), and one from a deeper interval in the sediment (0.5-1cm). Specimens S1 and S2 (top interval) show relatively stable Mg/Ca and Ba/Ca ratios along the 10-11 chamber stages measured (Figures 6A, C, E, and G), while Sr/Ca ratios slightly vary but do not show a clear trend (Figures 6B and F). The Mn/Ca ratios of these two specimens are low and relatively constant with the exception of chamber stages F-5 and F-6 in S1 (Figures 6D and H). On the other hand, specimen S3 (deep interval) shows an increase in Mg/Ca, Ba/Ca and Mn/Ca ratios from the oldest to the newest chamber (Figures 6I, K, and L) while Sr/Ca values do not show correlation with the chamber stage (Figure 6J).

6.4. Discussion

6.4.1. Trace element distribution

The specimens analysed were stained with Rose Bengal, which implies that they were alive at time of collection. This effectively excludes the presence of early diagenetic $MnCO_3$ or Mn-oxides on the calcitic tests. Moreover, the element/Calcium ratios were plotted against [27]Al-counts to detect the presence of Al-rich clay particles that could bias our analyses. In addition to carefully screening the elemental profiles for each ablation crater with respect to analyses time, we evaluated the calculated overall

concentrations for correlations with [27]Al-counts and omitted the suspect analytical results from further discussion. Although clay particles are also usually enriched in Mg, no correlation was found between foraminiferal Mg/Ca ratios and counts of [27]Al. The fact that we did find similar trends (positive correlation) in a limited number of ablation craters for Mn/Ca, Ba/Ca, and the [27]Al counts (Figures 3C and D) implies that Ba and Mn are probably more sensitive to contamination coming from clay particles due to their low test concentrations.

Table 3. Mg-based temperatures[a].

Chamber	No. specimen	Mg/Ca (mmol/mol)	Temperature (°C)
Top Interval (0.0-0.5 cm)	1	0.90	0.01
	2	0.87	-0.27
	3	1.17	2.35
	4	1.28	3.21
	5	0.90	-0.02
	6	1.57	5.05
	7	0.92	0.20
Deep Interval (0.5-1.0 cm)	8	1.29	3.27
	9	1.27	3.16
	10	1.03	1.23
	11	1.27	3.14
	12	1.36	3.77
Average			2.06
SD[b]			1.72

[a]Temperatures were calculated using the equation Mg/Ca = 0.90 exp(0.11*T) (Elderfield et al., 2006)
[b]SD= standard deviation that corresponds to inter-individual variability.

6.4.2. Inter-individual variation of Mg/Ca, Sr/Ca, Ba/Ca, and Mn/Ca

Variability in Mg incorporation between individuals (Table 2) is too large to be explained by analytical uncertainties alone (Figure 4A). The standard deviations shown in Table 2 (SD[a]), which reflect analytical plus intra-chamber variability, are smaller than the second set of standard deviations also shown in Table 2 (SD[b]), which indicate inter-individual variation. This implies that the in-between-test variability (inter-individual) is larger than the within-test variability (intra-individual). Post depositional dissolution of the carbonate test has to be discarded as source of inter-individual variability, since we analysed only living specimen sampled alive.
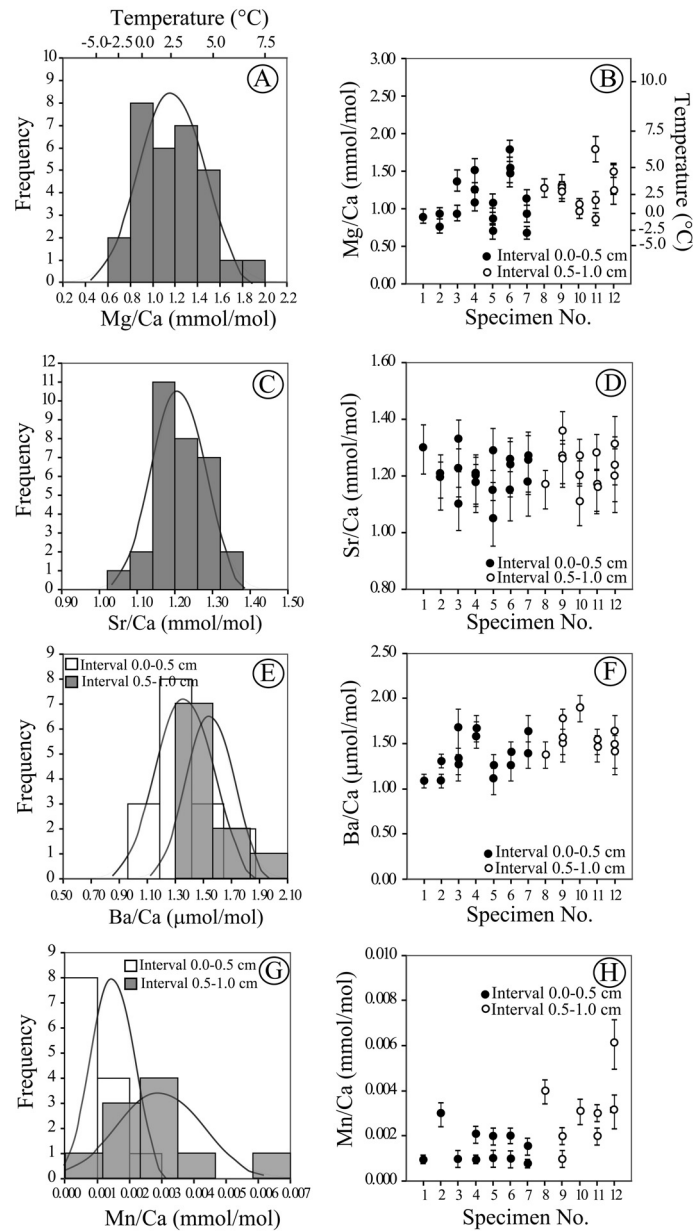
Figure 4. Trace element composition of 12 specimens of *Cibicidoides pachyderma*. A, C, E, and G correspond to histograms (frequency distributions) of Mg/Ca, Sr/Ca, Ba/Ca, and Mn/Ca, respectively. B, D, F, and H correspond to Mg/Ca, Sr/Ca, Ba/Ca, and Mn/Ca, respectively. For the intervals 0.0-0.5 cm (closed circles) and 0.5-1.0 cm (open circles). Mg/Ca based temperature was calculated using the equation: Mg/Ca = 0.90 exp(0.11*T) (Elderfield et al., 2006).

Specimens were collected from a station located at 550 m depth, thus temperature and salinity do not significantly vary over time. Consequently, changes in other parameters, such as the carbonate ion concentration $[CO_3^{2-}]$ must be responsible for the observed Mg variability. From the reaction-transport model proposed by Jourabchi et al. (2005) to interpret pH distributions in marine sediments, a change of ~600 μmol/kg in $[CO_3^{2-}]$ is calculated within the first centimeter. When using the ΔMg/Ca-$[CO_3^{2-}]$ calibration for *C. wuellerstorfi* from Raitzsch et al. (2008), we infer that the observed inter-individual variability in Mg/Ca ratios of 20% (standard deviation, SD[b], in Table 2) is equivalent to a change of only ~100 μmol/kg in $[CO_3^{2-}]$, which falls well within the pH model calculations for the first centimeter. This implies that specimens of *C. pachyderma* might migrate vertically and probably calcify at slightly different depths within the sediment. This could introduce the observed variability (Figures 4A and B). The lack of a clear difference between the specimens from the top sediment interval and the deep interval (Figures 4A and B) suggests that foraminifera do not necessarily reflect the depth level where they are collected with regard to Mg/Ca ratios.

Several authors (Eggins et al., 2003; Sadekov et al., 2008; Hathorne et al., 2009) have suggested a differential development of Mg/Ca banding within test walls of planktonic foraminifera as a cause for inter-individual Mg variation. Differences in the thickness of low and high-Mg layers between individuals might also contribute to the inter-individual variation found in *C. pachyderma*. The laser-ablation profiles of the umbilical side of three specimens of *C. pachyderma* (Figure 5) encompass all the calcite that was built during the life cycle of these individuals. Therefore, this is an ideal way of looking at variations within the foraminiferal test walls. From these profiles, intervals of high-Mg and low-Mg can be observed which might contribute to the inter-individual variation in this benthic species.

Using the equation Mg/Ca = 0.90 exp(0.11*T), proposed by (Elderfield et al., 2006) for *Cibicidoides* species, results in a Mg-based temperature of 2.1 ± 1.76°C. This calculated temperature is significantly lower than the actual "in situ" temperature of ~11°C. This implies that the measured Mg/Ca values are too low compared to the calibration used. There are two possible causes that could explain these results: 1) the laser ablation analyses resulted in too low values, or 2) the tests were already affected by other factors.

The results from the matrix matched calcite standard, the uncommonly homogeneous calcite crystal (Icelandspar), were compared to the laser-ablation analyses performed on the same standard during a

four-year period (Nobbe, 2008). The present analyses did not show any offset with respect to analyses performed in the past (Appendix, Figure D1). Other foraminiferal species analysed during this time interval did correspond with previously published calibrations (e.g. Dueñas-Bohórquez et al., 2009; Raitzsch et al., 2009; Dissard et al., 2009c). The laser-ablation measurements of the calcite standard (Icelandspar) were also compared to ICP-MS analyses but no significant deviations were found (Appendix, Figure D1). This implies that other factors rather than the laser-ablation procedure as such must be responsible for the observed low Mg/Ca ratios.
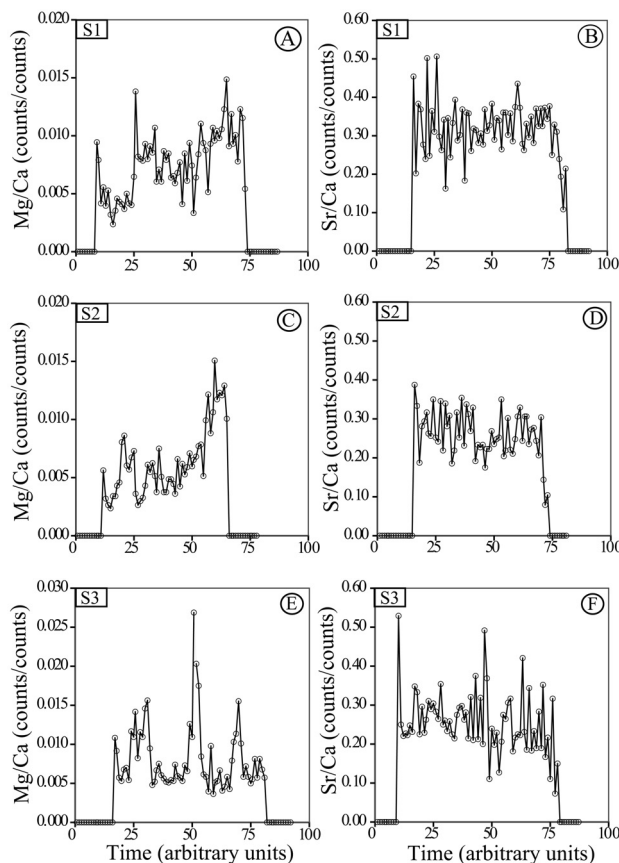
Figure 5. Laser ablation profiles of the umbilical side of three specimens of *Cibicidoides pachyderma*. A. and B. Mg/Ca and Sr/Ca (counts/counts), respectively, of specimen S1 collected from the top sediment interval (0.0-0.5cm). C. and D. Mg/Ca and Sr/Ca (counts/counts), respectively, of specimen S2 collected from the top sediment interval. E and F correspond to Mg/Ca and Sr/Ca (counts/counts), respectively, of specimen S3 collected from the deep sediment interval (0.5-1.0cm).

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Dissolution processes and cleaning procedures are discarded as source of Mg-loss in the foraminiferal calcite since all individuals measured were alive at the time of collection; therefore, there was no need of using extensive cleaning procedures. On the other hand, a possible source of contamination would increase rather than decrease the Mg values. Based on the Mg/Ca temperature calibration for *Cibicidoides* the expected Mg/Ca value for a temperature of ~11°C is approximately 3 mmol/mol, considerably higher than the average Mg/Ca value measured (1.15 ± 0.22; Table 2). If we consider changes in $[CO_3^{2-}]$ of the pore-water as the parameter responsible for this decrease in Mg/Ca, a change of ~200 µmol/kg in $[CO_3^{2-}]$ should be present to cause the low Mg/Ca values obtained here (according to Mg/Ca-$[CO_3^{2-}]$ equations for *Cibicidoides* species in Raitzsch et al., 2008). Such a large variation in $[CO_3^{2-}]$ of the pore-water sediments at this station is not plausible. Although without a more detailed local calibration, we cannot completely discard local chemical effects.

Alternatively, the observed offset might be due to cryptic speciation in *C. pachyderma*. This implies that the individuals measured here would be genetically different but morphologically identical to the *Cibicidoides* genus used in temperature calibrations. In benthic foraminifera, cryptic speciation has been identified in the genus *Ammonia* (Holzmann and Pawlowski, 2000) and the benthic foraminifera *Planoglabratella opercularis* (Tsuchiya et al., 2003). Hence, cryptic speciation in deep benthic foraminifera such as *C. pachyderma* cannot be discarded.

Using the same Mg-Ca/temperature calibration (Elderfield et al., 2006), we calculated that the Mg/Ca inter-individual variability found in *C. pachyderma* (20%; Table 2) potentially contributes 2°C to the apparent temperature variance (Table 2, Figure 4A). From these results, it is clear that in order to obtain reliable reconstructions of deep water temperatures, calculations have to be based on a sufficient number of individuals, which will depend on species, sediment depth distribution and changes in the carbonate chemistry within the sediment top layer.

The inter-individual variability found in Sr/Ca ratios corresponds to 4% of the average Sr/Ca value (Table 2; Figures 4C and D). In inorganic and biogenic calcite, the Sr/Ca ratio is known to increase with growth rate (Lorens, 1981; Nehrke et al., 2007; Tang et al., 2008; Kısakürek et al., 2008). The Sr variability observed might be caused by slight differences in the growth rate between individuals. A clear Sr/banding within the test walls of *C. pachyderma* seems to be absent (Figures 5B, D, and F).
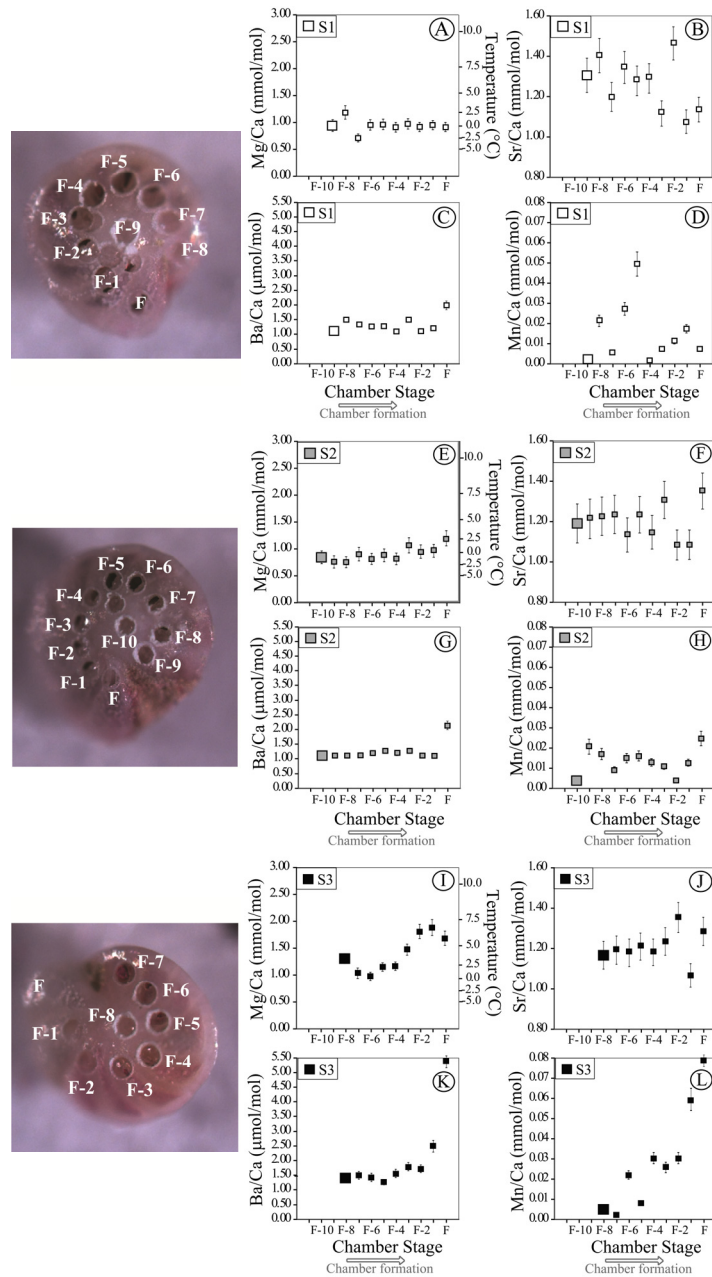
Figure 6. Trace element composition per chamber for three specimens of *Cibicidoides pachyderma*: S1 and S2 correspond to specimens found in the sediment interval 0.0-0.5 cm; S3 corresponds to a specimen found in the sediment interval 0.5-1.0 cm. A. Mg/Ca ratios; B. Sr/Ca ratios; C. Ba/Ca ratios; and D. Mn/Ca ratios. Enlarged squares in every plot correspond to the analysis of the umbilical side which encompasses all chambers.

The small inter-individual variability observed in Ba/Ca ratios from the two sediment intervals analysed (14 and 13% for the top and deep intervals, respectively; Table 2, Figures 4E, and F) might be explained by a microhabitat effect (migration). Barium concentration in pore water tends to increase 2 to 6 times in magnitude and sometimes even more, within the first centimeter in the sediments (McManus et al., 1998). This process is caused by the ongoing dissolution of barite due to seawater under-saturation with respect to $BaSO_4$, although the exact mechanisms involved are poorly understood (Schenau et al., 2001).The resulting Ba gradient commonly observed in pore-water of marine sediments should be recorded in the Ba composition of benthic foraminifera (Lea and Boyle, 1989). The overall higher foraminiferal Ba concentrations in the deeper sediment interval studied (Figure 4F) suggests that this is indeed the case. This implies that the calcite composition of this benthic foraminifera reflect the chemistry of pore-water at specific sediment depths and foraminifera tend to calcify at similar depths within the sediment. It is also clear from our results that deep benthic foraminifera slightly migrate, behaviour that is reflected in the small inter-individual variability within specimens belonging to the same sediment interval. Using only the Ba concentrations of the foraminifera from the shallow sediment interval, we calculated the values of Ba concentration in bottom-water, using the established D_{Ba} (0.37 ±0.06; Lea and Boyle, 1989) (Figure 7A). The result obtained suggests a slightly lower Ba concentration in bottom-water than the concentration based on extrapolating the GEOSECS data for the Bay of Biscay (Chan et al., 1977) (Figure 7A).

The D_{Ba} of *C. pachyderma* at this depth might be moderately smaller ($D_{Ba} \approx 0.30 \pm 0.06$) that the one proposed by Lea and Boyle (1989), although this is within the uncertainty limit. The inter-individual variability in Mn/Ca observed in the two sediment intervals analysed, after discarding suspect data (see section 4.1), is comparable with the analytical standard deviation (Table 2; Figure 4H). This implies that, in contrast to Mg, Mn is reliably recorded in the test carbonate of single individuals. The average Mn/Ca value calculated for the deeper sediment interval is slightly higher than the average Mn/Ca ratio obtained for the top sediment interval (Figure 4H). Pore-water Mn significantly increases between 1.50 and 4.00 cm depth in the sediment (Table 1, Figure 8A), while oxygen simultaneously disappears (Mouret et al., 2009). This implies that foraminifera might migrate and calcify slightly deeper in the sediments than where they were actually collected. The slightly different foraminiferal Mn/Ca averages found between the two studied sediment

intervals implies that the calcite composition of this benthic species reflects the pore-water Mn increase to some extent (Figure 4D).

6.4.3. Intra-individual variation in Mg/Ca, Sr/Ca, Ba/Ca, and Mn/Ca

Comparing the Mg/Ca ratios of chambers from specimens S1 and S2 (top interval; Figures 6A and E) shows no significant difference between test chambers during calcification. This indicates that pore-water chemistry, ontogenetic or biological effects (e.g. metabolic rates) did not influence Mg uptake in these two specimen. However, the specimen collected from the deepest interval (0.5-1cm) (S3) shows a significant difference in the Mg/Ca values between the last four chambers added and the previous ones (Figure 6I). Such a change can be explained by 1) a significant decrease in pore-water $[CO_3^{2-}]$ with depth within the sediments, or 2) a vital and/or ontogenetic effect. The first explanation is based on combining the modelled in-sediment decrease in $[CO_3^{2-}]$ (Jourabchi et al., 2005) with the measured effect of $[CO_3^{2-}]$ on Mg incorporation (Elderfield et al., 2006; Raitzsch et al., 2008; Yu and Elderfield, 2008). Since Mg incorporation increases at higher $[CO_3^{2-}]$, this would imply that calcification of the last Mg-enriched chamber stages occurred at the top sediment interval. The second explanation involves a ontogenetic and/or vital effect. A change in the metabolic rate (e.g. respiration) with growth potentially has an influence on the local carbonate chemistry around the foraminifer. However, this Mg pattern would also have to be observed in specimens S1 an S2 from the top interval, which is not the case.

The lack of intra-individual variation in the Sr/Ca ratios indicates that Sr is not affected by either pore-water chemistry, migration patterns or vital effect (Figures 4B, 5B, 5F, and 5J). Previous studies on planktonic and benthic foraminifera (Russell et al., 2004; Dueñas-Bohórquez et al., 2009; Chapter 4 in this book), however, showed positive correlations between the $[CO_3^{2-}]$ of the surrounding seawater and the foraminiferal Sr incorporation. Comparable changes in the Sr incorporation of *C. pachyderma* that might be triggered by $[CO_3^{2-}]$ variations are not observed (Figure 4D, 5B, 5F, and 5J). This means that differences in $[CO_3^{2-}]$ did not play a major role in the variation observed in Mg/Ca profiles, or the impact of $[CO_3^{2-}]$ on Sr is species specific and rather limited in this benthic species. Profiles of pore-water Ba from deep stations located in central and southern California margin, show that Ba concentrations rapidly increase within the first few centimeters (McManus et al., 1998).

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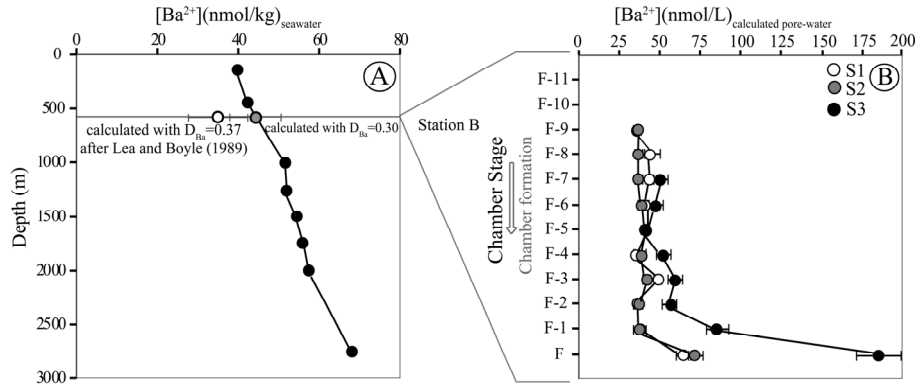
Figure 7. A. Seawater Ba concentrations according to the GEOSECS data base (closed circles); open circle corresponds to data calculated from the average Ba/Ca ratios of individuals of *Cibicidoides pachyderma* (present study) from the top sediment interval (0.0-0.5 cm) and DBa from Lea and Boyle (1989); grey circle corresponds to data calculated from the average Ba/Ca ratios of individuals of *C. pachyderma* (present study) from the top sediment interval (0.0-0.5 cm) and the new DBa=0.30 proposed here. B. Ba concentration of pore-water calculated using DBa = 0.30 and Ba/Ca ratios of single chambers of three specimens of *C. pachyderma*: S1 and S2 correspond to individuals found in the top-sediment interval (0.0-0.5 cm); S3 corresponds to individual collected in the deep sediment interval (0.5-1.0 cm).

The increase in Ba/Ca ratios observed in specimen S3 suggests that this specimen migrated into the sediment, similar to the behavior observed between the two groups compared in section 4.2 (Figure 4F). The newly established D_{Ba} of 0.30 together with the Ba/Ca ratios of single chambers of these three specimens were used to calculate the pore-water Ba concentration within the top centimeter of the station studied (Figure 7B). The reconstructed gradient suggests that individuals of *C. pachyderma* migrate deeper in the sediment at the later stage of their life cycle.

The Mn/Ca variation between chamber stages of the specimens from the sediment top-interval (S1 and S2) does not show a clear trend (Figures 6D and H) that can be matched to possible microhabitat or migration patterns. However, specimen S3, which was collected from a slightly infaunal habitat, shows higher Mn/Ca values in its final chamber stages (Figure 6L). This agrees with the typical Mn gradient found in pore-water. Calculating pore-water Mn concentration using the partition coefficient for Mn suggested by Lea (1999) (D_{Mn}= 2) and the Mn/Ca ratios measured in chambers of specimen S3, indicates values which are in line

with the Mn concentrations measured (Anschutz, pers. comm.; Figure 8B). Once more, this particular specimen probably calcified its last chambers (F-2, F-1, and F) at a greater in-sediment depth.

6.4.4. Impact of migration on Mg/Ca, Ba/Ca, and Mn/Ca

The variability found in Ba/Ca and Mn/Ca ratios within chambers of specimens of *C. pachyderma* agrees well with the typical Mn and Ba gradients in pore-water. For one of the specimens it suggests vertical migration towards deeper areas in the sediment at a later life cycle stage (Figures 5, 6, and 7). However, the gradual increase in Mg concentrations found in this specimen (S3, deeper interval: 0.5-1 cm) does not match the migration patterns indicated by the changes in Ba/Ca and Mn/Ca between chambers, assuming that the Mg/Ca change is related to differences in $[CO_3^{2-}]$. If the observed Mg/Ca variability was related to changes in pore water $[CO_3^{2}]$, this would imply an upward migration instead of the deeper habitat suggested by both Mn and Ba (see sections 4.2 and 4.3). A possible explanation for the observed increase in foraminiferal Mg/Ca ratios within the three final chambers of specimen 3 involves the presence of food cysts around specimens during early life stages. Living at the top of the sediment during a sudden episode of increased carbon flux to the sea floor such a cyst would decrease pH locally.
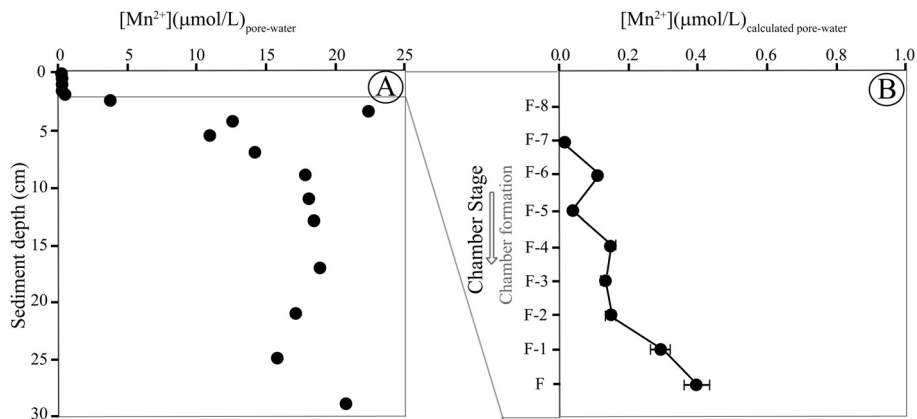
Figure 8. A. Mn concentration in the pore water of Station B, Bay of Biscay (Mouret et al., 2009). B. Mn concentration in the pore-water calculated from the Mn/Ca ratios of single chambers of specimen S3, collected in the deep sediment interval (0.5 -1.0 cm), and DMn suggested by Lea (1999).

The lower pH and thus $[CO_3^{2-}]$ in the microenvironment, would decrease the Mg incorporation (Elderfield et al., 2006; Raitzsch et al., 2008; Chapter 4 in this book). After the carbon has been consumed by the combined benthic fauna, the foraminifer moved deeper into the sediment, changing its microhabitat. Consequently, the pH and $[CO_3^{2-}]$ in the surrounding microenvironment would increase, leading to calcite with higher Mg/Ca ratios, and also higher Mn and Ba incorporation.

6.5. Conclusions

Inter and intra-individual variations in the trace element composition of tests of the deep benthic foraminifera *C. pachyderma* reflect changes in the chemistry of the pore-water. The Ba and Mn calcite composition of the test carbonate indicates vertical migration within the top few centimeters of the sediment. The lack of inter and intra-individual variation in the Sr/Ca ratios indicates that Sr is apparently not affected by either pore-water chemistry, migration patterns or vital effect in this benthic species. Intra-individual variation in Mg/Ca ratios cannot be explained by migration alone, whereas changes in Ba and Mn concentrations in the pore-water are more directly reflected in the test carbonate. The observed changes in Mg/Ca suggest that food (cysts) surrounding the foraminifera locally influenced the carbonate chemistry and thus Mg-uptake.

Acknowledgements

Chapter 7

Synopsis: Geochemical and biological impacts on trace and minor element incorporation in foraminiferal test carbonate

The reconstruction of past climates relies on so-called proxies. Proxies are parameters that can be measured in sediment or ice records that have responded systematically to changes in important but unmeasurable variables in the past, such as temperature and salinity. Several of these proxies are based on the incorporation of trace and minor elements into foraminiferal tests. Although both field and controlled growth experiments contributed greatly over the last few decades to more reliable foraminiferal proxies, calibrations remain essentially empirical. The robust interpretation and application of proxy relationships in the geological past, however requires a fundamental understanding of the proxies themselves, based on physical, chemical and biological concepts. This is important since the relations underlying the empirical proxy calibration potentially changed over geological time, whereas the underlying thermodynamics remained the same. This implies that it is essential to determine the geochemical and physiological controls on foraminiferal trace and minor element incorporation and integrate these new findings with established calibrations. For other applications, such as a salinity proxy based on combining δ^{18}O-Mg/Ca, it is crucial to eliminate any biases to increase precision.

The aim of the research presented in this thesis is to improve our understanding of the effect of several geochemical and biological factors that have a relevant but hitherto unclear impact on foraminiferal proxies. This research focused on the influence of sea and pore-water chemistry, the impact of inter-individual (between individuals), and within individual (ontogenetic) variability on the incorporation of trace (Ba, Mn) and minor (Mg, Sr) elements in foraminiferal test carbonate. In order to separate the individual impacts of chemical and biological parameters, laboratory culture experiments were used. The advantage of using controlled environments lies in the possibility to vary certain seawater parameters, while keeping others constant. Using this approach, the isolated effects of seawater salinity, carbonate parameters and saturation state on foraminiferal trace and minor element incorporation were determined. Four different species of living planktonic and benthic foraminifera were selected based on the following criteria: 1) species widely used in paleoceanographic and paleoclimate studies for sea surface/bottom water temperature reconstructions; 2) species with contrasting calcification strategies; 3) species that showed high growth and survival rates under laboratory conditions.

Chemical parameters of the seawater

The following seawater parameters were targeted: salinity, calcium carbonate saturation state (Ω), carbonate ion concentration ($[CO_3^{2-}]$) and calcium ion concentration ($[Ca^{2+}]$). Salinity is the parameter responsible for the changes in Mg incorporation in the planktonic foraminifer *Globigerinoides sacculifer* (under a constant temperature) while Ω apparently does not have any influence on this minor element (**Chapter 2**). The mechanism responsible for the impact of salinity on Mg/Ca ratios is not well understood. However, it is suggested that the salinity effect on Mg incorporation is a result of an increase in the seawater $[Mg^{2+}]$. This increase will be reflected in the parent solution used at the calcification site. It is assumed here that the amount of Mg^{2+} extracted from the parent solution does not vary and the foraminifer continues to use the same amount of energy in order to lower $[Mg^{2+}]$. This implies a strong biological control on Mg incorporation, which partly reflects the energy invested.

When investigating the impact of seawater $[CO_3^{2-}]$ on the incorporation of Mg, the responses observed in planktonic (*G. sacculifer*) and shallow benthic foraminifera (*A. tepida* and *H. depressa*) differ (**Chapters 2 and 4**). While Mg incorporation in the planktonic species does not correlate with seawater $[CO_3^{2-}]$ (**Chapter 2**) or, in some cases negatively correlates (e.g. Russell et al., 2004), the Mg content in the tests of shallow benthic species shows the opposite trend (**Chapter 4**). This indicates that the biomineralization mechanisms involved in the uptake and incorporation of Mg into the calcitic tests respond differently under similar seawater chemical conditions, depending on the foraminifer (type) studied. Similarly, incorporation of Sr in the planktonic species studied (*G. sacculifer*) is significantly affected by seawater $[CO_3^{2-}]$ (**chapter 2**), while shallow benthic species do not show a relevant change in Sr over a similar range. In inorganic (Lorens, 1981; Nehrke et al., 2007; Tang et al., 2008) and biogenic calcite of planktonic species (Kısakürek et al., 2008), the Sr/Ca ratio is known to increase with growth rate, and a higher $[CO_3^{2-}]$ increases planktonic foraminiferal shell weight and possibly calcification rates (Bijma et al., 1999). The growth rates of *A. tepida* and *H. depressa* (referred in **Chapter 4** as number of chambers added during culturing experiments) are not appreciably affected by changes in $[CO_3^{2-}]$. This might be the reason that no major changes in Sr incorporation were observed in these two benthic species. However, the

moderate response of the calcification rate in the benthic foraminifera studied indicates that their calcification is under strict biological control.

The different responses in growth rates observed in planktonic and shallow benthic species are of great importance when looking at the impact of ocean acidification on marine calcifiers. The results shown here imply that shallow benthic foraminifera *A. tepida* and *H. depressa* might not be severely affected by the ongoing decrease of pH in the oceans (Ocean acidification), while calcification in the planktonic species *G.sacculifer* might be more susceptible.

From the results obtained in **Chapters 3 and 4**, it is inferred that Ω is not directly responsible for the observed changes in Mg and Sr incorporation in the shallow benthic foraminifera *A. tepida* and *H. depressa* but rather that seawater $[CO_3^{2-}]$ and $[Ca^{2+}]$ are the controlling parameters. From these two parameters, $[Ca^{2+}]$ has a significant impact on the incorporation of both minor elements. This observation implies the presence of two independent mechanisms involved in biomineralization.

Biomineralization

The inferred two independent mechanisms involved in controlling the internal calcification reservoirs are:
1) a calcium-concentrating mechanism, which increases the internal $[Ca^{2+}]$.
2) a mechanism in which bicarbonate from the seawater is transported to a DIC-reservoir, likely to be converted afterwards to increase internal $[CO_3^{2-}]$.

The first mechanism might be involve in divalent-cation (Mg^{2+}, Sr^{2+}) discrimination. This discrimination against Mg by a divalent cation pump probably determines Mg isotopic fractionation as well in low-Mg foraminifera. The second mechanism might be associated with exchange of negatively charged ions to keep electro-neutrality inside the vacuolized seawater. The mechanism of HCO_3^- transport allows ions with the same charge and a similar size to enter the inorganic carbon pool such as $B(OH)_4^-$. According to Bentov et al. (2009), transport of seawater via fluid phase endocytosis may account for most of the ions supplied to the calcification site in the shallow benthic foraminfer *Amphistegina lobifera*. This implies that no channels or pumps would be needed in order to vary the chemistry of the calcifying parent solution. However, the contrasting

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results on Mg incorporation obtained in **Chapters 3** and **4,** when $[CO_3^{2-}]$ and $[Ca^{2+}]$ are varied independently, clearly indicate the presence of two distinct ion-transport mechanisms. Moreover, without such channels low Mg calcite, and its Mg- isotope depletion, would be impossible.

Biological and microenvironment parameters

The inter-individual and ontogenetic effects on planktonic foraminiferal Mg and Sr incorporation shown in **Chapter 5** indicate the need to incorporate these parameters in existing calibrations. The results obtained here demonstrate the presence of a significant influence of inter-individual and ontogenetic variability that should be included in Mg/Ca-temperature calibrations. Quantifying the magnitude of these effects is of great importance since, for instance, the use of inter-individual variability to reconstruct seasonality is starting to increase (Sadekov et al., 2008).

Trace element composition (Ba and Mn) of tests of deep-sea benthic foraminifer *Cibicidoides pachyderma* reflects the chemistry of the microenvironment (pore-water) and migration patterns as shown in **Chapter 6**. This indicates that foraminifera are potentially indicators of the chemistry of the surrounding (micro) environment. By combining microhabitat preference (including migration) of deep benthic foraminifera with geochemical cycling, it might be possible to develop proxies for the reconstruction of pore-water gradients in the past. Laboratory culture studies are needed in order to accurately quantify incorporation of redox-sensitive elements into foraminiferal tests, establish their partition coefficient and, dependence on other factors such as pore-water carbonate chemistry.

Outlook

The increase in atmospheric CO_2 causes changes in the carbonate system of the surface ocean. One of these changes involves a decrease in pH of the oceans (Zeebe and Wolf-Gladrow, 2005). These variations in the chemistry of the ocean may have significant consequences for biomineralization processes in foraminifera. An increase in atmospheric CO_2, will increase ocean CO_2 leading to a decrease in the calcite saturation state of the (surface) seawater, Ω. Calcification processes have been found to be affected by changes in $[CO_3^{2-}]$ rather than Ω (**Chapters 2 and 4**), with a decrease in calcite formation at higher CO_2 that is more pronounced in planktonic species than in shallow benthic species

(Kısakürek et al., 2008; Bijma et al., 1999; **Chapters 2 and 3**, present research). However, since foraminifera apparently take up both carbonate and bicarbonate from seawater, ocean acidification does not necessarily have an adverse effect on their ability to biomineralize. The foraminifera basically take up total DIC, which internally is changed in carbonate ions through proton pumps. The higher total DIC available as a consequence of ocean acidification would therefore enlarge the available carbon for biomineralization. However, the conversion of bicarbonate into carbonate needed for calcification costs energy. In **Chapter 2,** the observed correlation between Mg incorporation and salinity outlined the limited flexibility of foraminifera to adapt their spending of energy for biomineralization. The ability of foraminifera to adapt to future ocean acidification is thus difficult to predict a priori.

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Samenvatting

Sinds de industriële revolutie heeft de grootschalige uitstoot van CO_2 zowel het klimaat als de oceaanchemie beïnvloed. Om de effecten hiervan in de toekomst te kunnen voorspellen worden modellen gebruikt van het klimaat en oceaan systeem op Aarde. Zulke modellen worden geverifieerd door middel van reconstructies van vroegere natuurlijke veranderingen. Deze reconstructies zijn gebaseerd op het toepassen van zogenaamde proxies, die meetbare variabelen in het fossiele archief met parameters, zoals temperatuur en zoutgehalte, in verband brengen. De chemische samenstelling van het carbonaat waarmee foraminiferen hun schaaltjes vormen is een belangrijke variabele die wordt toegepast als proxie. Veel van de processen betrokken bij de inbouw van sporenelementen in foraminiferenschaaltjes zijn echter nog onbekend. Experimenten in het laboratorium en de analyse van levende, uit hun natuurlijk milieu verzamelde, organismen zijn nodig om deze onbekende factoren te bestuderen en op deze manier de proxies en dus ook de (klimaats)reconstructies te verbeteren.

Foraminifera zijn eencellige organismen die tot het Koninkrijk Protista behoren. De belangrijkste kenmerken van dit taxon zijn de aanwezigheid van een sterk georganiseerd cytoplasma met een netwerk van pseudopodiën en, de aanwezigheid van een schaal, test, die, afhankelijk van de foraminiferen groep, uit biogeen calciumcarbonaat (kalk), sedimentdeeltjes of organisch materiaal (polysacchariden) kan bestaan. Foraminiferen komen frequent voor, zowel met een benthische als met een planktonische leefwijze. De benthische foraminiferen zijn al vroeg in de evolutie ontstaan en worden reeds gevonden in Cambrische afzettingen (rond 525 Ma), terwijl planktonische foraminiferen ongeveer 200 Ma geleden voor het eerst ontstonden. Sindsdien zijn zowel benthische als planktonische foraminiferen min of meer continue aanwezig in het fossiele archief. Dit heeft inmiddels geleid tot meer dan 10.000 soorten, waarvan slechts 40-50 planktonische. De wereldwijde aanwezigheid van foraminiferen in het mariene milieu, hun hoge fossilisatie potentieel, en hun lange, vrijwel continue, aanwezigheid in het geologische archief, maken dat foraminiferen het meest gebruikte instrument zijn geworden voor de reconstructie van het klimaat en de oceaan in het geologische verleden.

Uitdagingen verbonden aan foraminiferale proxies: Deel I

Voor de toepassing in palaeoklimatologisch en palaeoceanografisch onderzoek zijn verschillende proxies ontwikkeld, gebruikmakend van de chemische samenstelling van foraminiferenschaaltjes gekoppeld aan specifieke processen: fysische en chemische proxies (b.v. Mg, Sr, $\delta^{11}B$, $\delta^{18}O$), biologische productiviteit proxies (b.v. Ba, Cd, $\delta^{13}C$), en proxies voor vroege diagenetische processen (b.v. Mn). De laatset tien jaar is het echter steeds duidelijker geworden dat deze foraminiferen proxies vaak door meer dan één oceaanparameter tegelijkertijd worden beïnvloed. Dit maakt hun interpretatie op zijn minst uitdagend (Nürnberg et al., 1996a en b; Lea et al., 1999; Ferguson et al., 2008).

De inbouw van Mg in de calcium-carbonaat schaaltjes van foraminiferen wordt vaak gebruikt voor de reconstructies van de temperatuur van zeewater waar deze foraminiferen leefden (Nürnberg et al., 1996a en b; Rosenthal et al., 1997). In combinatie met de stabiele zuurstof-isotoop verhouding ($\delta^{18}O$) van dat zelfde carbonaat kan dan vervolgens het zoutgehalte van dat water uitgerekend worden (Rohling, 2000). Veranderingen in de inbouw van Sr in het carbonaat worden deels toegeschreven aan veranderingen in de Sr/Ca ratio van het zeewater (Stoll et al., 1999), deels aan veranderingen in pCO$_2$ (Rosenthal et al., 1997) en deels aan temperatuur (Reichart et al., 2003). Het is inmiddels echter ook duidelijk dat andere zeewaterparameters zoals het zoutgehalte en de zeewater carbonaatchemie, een extra invloed hebben op de inbouw van zowel Mg als Sr (Russell et al., 2004; Ferguson et al., 2008). Het doel van de eerste twee hoofdstukken van dit proefschrift is het onafhankelijk bepalen van de effecten van de meest relevante parameters van de zeewatercarbonaat chemie op de inbouw van Sr en Mg om op deze manier hun gebruik als proxies te verbeteren.

Het effect van het zoutgehalte van zeewater op de inbouw van Mg en Sr in het carbonaat van de schaaltjes van de planktonische foraminifeer *Globigerinoides sacculifer* (sensu stricto) wordt gekwantificeerd in **Hoofdstuk 2**. Deze organismen werden met behulp van scuba-duiken levend verzameld en in het laboratorium gekweekt onder verschillende, strikt gecontroleerde omstandigheden. Hoewel effect van zeewatertemperatuur belangrijker is dan dat van het zoutgehalte, resulteert een verandering van 4 saliniteit eenheden in een afwijking van 1°C. Ondanks dat dit effect relatief gering is kan het niet verwaarloosd worden bij het combineren van Mg/Ca-temperatuur reconstructies met de $\delta^{18}O$ waarden van foraminiferen om saliniteit uit te rekenen. Deze studie toont bovendien aan dat het zoutgehalte zelf de belangrijkste parameter is

die, bij constante temperatuur, de inbouw van Mg bepaald en niet de hiervan afhankelijke verzadigingsgraad van het zeewater ten opzichte van calcium carbonaat (Ω). Het mechanisme, verantwoordelijk voor het effect van saliniteit op de inbouw van Mg, is niet goed begrepen. De meest waarschijnlijke verklaring is dat de absolute concentratie van Mg in zeewater mede bepalend is. Een verhoging van de absolute Mg concentratie wordt via vacuolisatie doorgegeven aan het interne reservoir met elementen die uiteindelijk in het carbonaat van de foraminiferen schaaltjes terecht komen. Deze verklaring is erop gebaseerd dat foraminiferen voor het vormen van hun carbonaatschaaltjes een gelimiteerde hoeveelheid energie tot hun beschikking hebben en daardoor ook beperkt zijn in de hoeveelheid Mg^{2+} die ze uit hun calcificatie reservoir kunnen pompen. Dit mechanisme impliceert een sterke fysiologische controle op de inbouw van Mg. In tegenstelling tot Mg wordt de inbouw van Sr wel in belangrijke mate bepaald door de zeewater Ω, met een toename in Sr van 0.10 mmol/mol per 100 μmol/kg $[CO_3^{2-}]$, terwijl het zoutgehalte zelf juist geen effect heeft op de inbouw van dit sporenelement.

De carbonaatverzadigingsgraad van zeewater, Ω, controleert de neerslag en het opnieuw oplossen van calciumcarbonaat ($CaCO_3$). Op deze manier speelt het een belangrijke rol in de mondiale koolstofkringloop. Omega is functie van de calcium en carbonaatconcentraties van zeewater ($[Ca^{2+}]$ en $[CO_3^{2-}]$, respectievelijk) en de temperatuur. Om het effect van Ω onafhankelijk via $[Ca^{2+}]$, $[CO_3^{2-}]$ en de andere carbonaatparameters van het zeewater te scheiden, werden experimenten in het laboratorium uitgevoerd. Twee contrasterende benthische foraminiferen soorten werden hiervoor gebruikt. Terwijl beide soorten in ondiep water leven bouwt de ene soort (*Heterostegina depressa*) kalkschaaltjes met hoge Mg concentraties en beschikt over symbionten, terwijl de andere soort (*Ammonia tepida*) schaaltjes maakt met lage Mg concentraties en leeft zonder symbionten. In **Hoofdstukken 3 en 4**, worden de effecten van veranderingen in Ω op de inbouw van Mg en Sr in benthische foraminiferen schaaltjes via $[Ca^{2+}]$ en $[CO_3^{2-}]$ onafhankelijk van elkaar onderzocht. De resultaten van deze experimenten tonen aan dat niet Ω zelf verantwoordelijk is voor de waargenomen veranderingen in de inbouw van Mg en Sr in ondiepe benthische foraminiferen *A. tepida* en *H. depressa*, maar dat $[CO_3^{2-}]$ en $[Ca^{2+}]$ de bepalende parameters zijn. Van deze twee parameters heeft $[Ca^{2+}]$ de meeste invloed op de inbouw van zowel Mg als Sr. De verschillende effecten van veranderingen in $[CO_3^{2-}]$ en $[Ca^{2+}]$ en verschillen tussen Mg arm en Mg rijk carbonaat betekenen dat er waarschijnlijk ook twee onafhankelijke mechanismen betrokken zijn bij

biomineralizatie in foraminiferen. Op de eerste plaats een mechanisme dat actief [Ca^{2+}] binnen de cel verhoogt en op de tweede plaats een mechanisme waarin bicarbonaat vanuit het zeewater naar binnen wordt gepompt. Binnen de cel wordt dat bicarbonaat vervolgens waarschijnlijk omgezet in [CO_3^{2-}], om de verzadigingsgraad te verhogen. Het eerste mechanisme beïnvloedt de inbouw van Sr en Mg door verschillen in de mate waarin onderscheid gemaakt wordt tussen tweewaardig-kationen (Ca^{2+}, Mg^{2+}, Sr^{2+}). Het tweede mechanisme beïnvloedt de inbouw van Sr en Mg omdat als protonen vanuit de cel naar het zeewater gepompt worden om het bicarbonaat om te zetten naar [CO_3^{2-}] tegelijkertijd, via uitwisseling van negatief geladen ionen, de elektro-neutraliteit behouden moet worden.

Uitdagingen van foraminiferale proxies: Deel II

De inbouw van sporenelementen en isotopen in het carbonaat van de foraminiferenschaaltjes is niet alleen een functie van de chemische en fysische parameters waar de foraminifeer leeft, maar wordt ook bepaald door complexe fysiologische processen. Doordat foraminiferen levende organisme zijn hebben tal van processen invloed op de chemische samenstelling van de schaaltjes. Deze processen worden vaak samen genomen onder de noemer "vitale effecten", daarbij verwijzend naar de onderliggende biologische achtergrond. (Nürnberg et al., 1996a; Erez, 2003, Eggins et al., 2003; Sadekov et al., 2005; Bentov en Erez, 2006). Het doel van **Hoofdstuk 5** is om verschillen in Mg en Sr inbouw tussen individuen en binnen individuen, de ontogenetische effecten, te bepalen in de planktonische foraminifeer *Globigerinoides sacculifer*. Door deze foraminiferen onder strikt gecontroleerde zeewateromstandigheden te laten groeien is er ook gekeken worden naar de onderliggende processen. Als we de Mg inbouw in het carbonaat van foraminiferen omrekenen naar temperatuur, dan resulteren de waargenomen verschillen tussen de individuele schaaltjes in een 2 to 3°C variabiliteit. Binnen een foraminiferenschaaltje zorgen verschillen in de Mg inbouw per kamer voor een schijnbare daling van ~1°C in de op Mg inbouw gebaseerde temperatuur per kamer (van het oudst aan het nieuwst). Een dergelijk ontogenetische effect op de inbouw van Mg beïnvloedt daardoor temperatuursreconstructies. Daarom is een nieuwe empirische formule afgeleid uit de metingen aan de individuele kamers: Mg/Ca = (0.55-0.0001*MSD)$e^{0.089*T}$, waarbij MSD staat voor de grootte van de schelp.

De chemische samenstelling van het carbonaat van benthische foraminiferenschaaltjes is een afspiegeling van de omstandigheden

waaronder ze dit carbonaat vormen en dus ook hun microhabitat voorkeur. De chemie van het poriewater wordt grotendeels bepaald door vroeg diagenetische processen. Benthische foraminiferen die in het sediment hun schaaltjes vormen gebruiken dit poriewater om hun carbonaat te vormen. De inbouw van sporenelementen in dit carbonaat is daardoor een functie van de vroeg diagenetische processen. Voor **Hoofdstuk 6** is de chemische samenstelling van individuele benthische diepzee foraminiferen (*Cibicidoides pachyderma*) vergeleken uit oppervlakte sedimenten van de Golf van Biskaje. Vergelijkingen tussen individuen en binnen individuen laten verschillen zien die overeen komen met de bekende veranderingen in de chemie van het bodem- en poriewater. Verschillen in de inbouw van Ba en Mn in het carbonaat geven aan dat de foraminiferen verticaal migreren binnen de bovenste paar centimeter van het sediment en ook hun carbonaat op verschillende dieptes in het sediment vormen. De relatief constante inbouw van Sr suggereert dat Sr niet, of slechts in geringe mate, wordt beïnvloed door verschillen in poriewater chemie. De geobserveerde verschillen in de inbouw van Mg binnen en tussen individuen hoeven niet alleen door migratie verklaard te worden omdat zowel poriewater chemie als ontogenetische processen hier door elkaar lopen.

Implicaties

De huidige stijging van CO_2 in de atmosfeer verandert het carbonaat systeem aan het oceaanoppervlak, wat merkbaar is door een daling van de pH (Zeebe en Wolf-Gladrow, 2005). Dit kan belangrijke gevolgen hebben voor de biomineralizatie. De opname van CO_2 door de oceaan zorgt voor een daling van de carbonaatverzadigingsgraad van het zeewater, Ω. De vorming van carbonaat bleek met name beïnvloed te worden door $[CO_3^{2-}]$ in plaats van door Ω (**Hoofdstukken 2 en 4**). De carbonaatvorming neemt in planktonische foraminiferen bij hogere CO_2 meer af dan in ondiep benthische soorten (Kısakürek et al., 2008; Bijma et al., 1999; **Hoofdstukken 2 en 3**). Aangezien foraminiferen blijkbaar totaal opgelost koolstof opnemen uit het zeewater heeft verzuring van de oceaan niet noodzakelijk een negatief effect op hun vermogen om kalkschaaltjes te bouwen. Het totaal opgenomen koolstof wordt in het protoplasma omgezet naar carbonaat door protonen naar buiten te pompen. De hogere CO_2 in de oceaan verhoogt daardoor het beschikbare koolstof voor biomineralizatie. Echter het kost energie om bicarbonaat naar carbonaat ionen om te zetten, nodig voor de vorming van calciumcarbonaat. De in **Hoofdstuk 2**, waargenomen correlatie tussen de

inbouw van Mg en zoutgehalte suggereert dat de foraminiferen een beperkt energie budget tot hun beschikking hebben voor biomineralizatie. Het vermogen van foraminiferen zich aan te passen aan toekomstige verzuring van de oceaan is dus moeilijk om a priori te voorspellen.

Resumen

Desde el inicio de la revolución industrial, la producción masiva de CO_2 ha afectado no sólo el clima en el ámbito mundial sino también la composición química del océano. Con el fin de predecir el cambio climático en el planeta, la comunidad científica fundamenta sus conocimientos en modelos numéricos. La precisión de tales modelos se puede verificar través de la reconstrucción de cambios climáticos del pasado. Estas reconstrucciones se basan a su vez en proxies (indicadores), los cuales relacionan variables que se pueden medir en el registro fósil con parámetros físicos y químicos del medio ambiente. Uno de los proxies más comunes en estudios paleoceanográficos consiste en cuantificar la incorporación de elementos químicos en carbonatos biogénicos. Por ejemplo, la incorporación de elementos traza y menores en conchas de foraminíferos pueden reflejar cambios en parámetros como la temperatura de la superficie del mar y la composición química del océano. Aunque la incorporación de elementos en conchas de foraminíferos es una herramienta muy importante en la reconstrucción de características oceanográficas del pasado, muchos de los aspectos que controlan este proceso son aún desconocidos.

El empleo de experimentos de laboratorio y análisis de individuos vivos pueden contribuir a identificar los procesos de biomineralización en foraminíferos y así mismo, los efectos que diferentes parámetros físicos y químicos del océano ejercen sobre la composición química de las conchas que estos organismos producen.

Los foraminíferos son organismos unicelulares que pertenecen al Reino Protista. Su protoplasma está diferenciado en un endoplasma y un ectoplasma, del cual emergen pseudópodos retráctiles que el organismo usa para la locomoción, captura de presas y formación de su esqueleto calcáreo (concha). Este esqueleto intraectoplásmico es la característica más sobresaliente de los foraminíferos, y el motivo de que sean susceptibles de fosilizar con relativa facilidad. El esqueleto está constituido por cámaras interconectadas por poros llamados forámenes (foramina) que, además, dan el nombre al phylum. La concha de los foraminíferos puede estar constituida por diferentes elementos de acuerdo con el grupo al que pertencen: carbonato de calcio de origen biológico (calcárea), usando partículas de sedimento (aglutinada) o con base en compuestos orgánicos (polisacáridos). Los foraminíferos presentan dos modos de vida claramente diferenciados: bentónico y planctónico, dependiendo de si

viven en el sedimento o en la columna de agua, respectivamente. Con alrededor de 10000 especies conocidas, los foraminíferos bentónicos presentan una alta diversidad y sus fósiles son los más antiguos, ya que los primeros registros datan del Precámbrico (aproximadamente 525 Ma). Por el contrario, los foraminíferos planctónicos son mucho menos diversos (con alrededor de 40 especies modernas) y presentan un menor registro geológico, con los primeros fósiles del período Jurásico (200 Ma). De esta manera, el phylum Foraminifera suele considerarse como el más importante de los grupos de microfósiles marinos debido a que son organismos muy abundantes en los sedimentos marinos y presentan una gran diversidad de especies. Lo anterior les confiere una gran utilidad en estudios de tipo paleoceanográfico y paleoclimatológico.

Desafíos asociados a proxies de foraminíferos: Primera parte

Diferentes proxies han sido identificados hasta el momento y son usados frecuentemente en investigación paleoceanográfica con foraminíferos, tales como: proxies físicos y químicos (e.g. Mg, Sr, $\delta^{11}B$, $\delta^{18}O$), proxies indicadores de productividad (e.g. Ba, Cd, $\delta^{13}C$), y proxies diagenéticos (e.g. Mn). Durante los últimos años, se ha demostrado que los proxies de foramíniferos son afectados frecuentemente por más de un parámetro oceanográfico a la vez, lo cual dificulta aún más su interpretación (Nürnberg et al., 1996a and b; Lea et al., 1999; Ferguson et al., 2008).

La incorporación de magnesio (Mg) en conchas calcíticas de foraminíferos es usada frecuentemente en la reconstrucción de la temperatura superficial del océano (Rosenthal et al., 1997). El magnesio (Mg) también se emplea en la reconstrucción de salinidad en conjunto con los isótopos estables de oxígeno de las conchas de foraminíferos (Rohling, 2000). Asimismo, las variaciones en la incorporación de estroncio (Sr) en foraminíferos son atribuidas a dos factores: en parte a cambios en la proporción Sr/Ca del agua de mar y en parte a cambios en pCO$_2$ (Stoll et al., 1999). Sin embargo, estudios recientes han demostrado que otros parámetros fisico-químicos del agua de mar, tales como la salinidad y la composición de carbonatos, podrían tener un impacto adicional en la incorporación de Mg y Sr, cuyo efecto no ha sido evaluado. Lo anterior podría alterar las calibraciones ya establecidas (Russell et Al., 2004; Ferguson et Al., 2008). Por lo tanto, el objetivo de los primeros dos capítulos de esta tesis es determinar el impacto de los parámetros más

relevantes de la química del agua de mar en la incorporación de estos proxies, evaluando cada uno de ellos por separado.

En el **Capítulo 2** de esta tesis se cuantificó el efecto de la salinidad en la incorporación de Mg y Sr en conchas del foraminífero planctónico *Globigerinoides sacculifer* (*sensu stricto*). Este foraminífero fue cultivado en el laboratorio bajo diferentes condiciones ambientales. Aunque el efecto de la temperatura es más importante que el efecto de la salinidad, un cambio de 4 unidades en la salinidad es equivalente a un cambio de 1°C en la temperatura, la cual es calculada con base en la composición de Mg/Ca en las conchas de los foraminíferos ($T_{Mg/Ca}$). Si bien este efecto de la salinidad en la incorporación de Mg es secundario, cuando se emplea $T_{Mg/Ca}$ en combinación con isótopos de oxígeno ($\delta^{18}O$) en conchas de foraminiferos, se encontró que el efecto de la salinidad debe tenerse en cuenta. Este estudio también demuestra que la salinidad es el parámetro principal que controla la incorporación de Mg dentro del intervalo del estado de saturación de la calcita del agua de mar (Ω) estudiado (5,25 y 6,50; $[CO_3^{2-}]$ entre 218 y 270 µmol/kg) a una temperatura constante de 26°C.

Durante el desarrollo de esta investigación, se hicieron algunos avances en la comprensión del mecanismo responsable del efecto de la salinidad sobre la proporción Mg/Ca en conchas de *G. Sacculifer*; sin embargo, éste no pudo ser dilucidado en su totalidad. Como explicación plausible, se sugiere que el efecto de la salinidad en la incorporación de Mg es el resultado de un aumento en la concentración de iones de magnesio ($[Mg^{2+}]$) en el agua de mar. Sesugiere que este incremento en $[Mg^{2+}]$ se puede detectar en la solución utilizada como materia prima en el área de calcificación. Por lo tanto, dado que la cantidad de Mg^{2+} extraído de esta solución no varía, se asume que el foraminífero continúa utilizando la misma cantidad de energía para reducir $[Mg^{2+}]$ en la solución. Esta observación evidencia un control biológico importante en la incorporación de Mg en las conchas de este foraminífero. Por otra parte, Ω parece ser el principal control durante la incorporación de Sr en conchas de este foraminífero planctónico (0,10 mmol/mol por cada 100 µmol/kg de incremento en $[CO_3^{2-}]$), mientras que no se observó ningún efecto de la salinidad en la incorporación de este elemento traza. Omega (Ω) controla la formación y la disolución de calcita ($CaCO_3$); de esta forma, este parámetro cumple un papel importante en el ciclo global del carbono. Omega depende a su vez de las concentraciones de calcio y carbonato en el agua de mar ($[Ca^{2+}]$ y $[CO_3^{2-}]$, respectivamente). Por lo tanto, con el fin de separar el efecto de Ω del producido por $[Ca^{2+}]$, $[CO_3^{2-}]$ y los demás

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parámetros de carbonato del agua de mar, se llevaron a cabo varios experimentos de laboratorio con dos especies de foraminíferos bentónicos de aguas someras: *Heterostegina depressa* (calcita con un alto contenido de Mg, asociado a simbiontes) y *Ammonia tepida* (calcita con un bajo contenido de Mg, sin simbiontes). En los **Capítulos 3 y 4** se determinaron, en forma independiente, los efectos de los iones $[Ca^{2+}]$ y $[CO_3^{2-}]$ del agua de mar en la incorporación de Mg y Sr en la calcita de foraminíferos bentónicos. Los resultados de estos experimentos indican que Ω no es directamente responsable de los cambios observados en la incorporación de Mg y Sr en las conchas de *A. tepida* y *H. depressa*, sino que $[CO_3^{2-}]$ y $[Ca^{2+}]$ son los parámetros que controlan la incorporación de estos elementos. De estos dos parámetros, $[Ca^{2+}]$ tiene un impacto significativo en la incorporación de Mg y Sr. Estos resultados sugieren la presencia de dos mecanismos independientes implicados en biomineralización: 1) un mecanismo encargado de acumular calcio, el cual incrementa $[Ca^{2+}]$ en el interior de la célula, y 2) un mecanismo en el que el bicarbonato del agua de mar $([HCO_3^{-}]$ es transportado internamente a un depósito de carbono inorgánico disuelto (CID). Es posible que el primer mecanismo este involucrado en el transporte selectivo de cationes divalentes (Mg^{2+}, Sr^{2+}), mientras que el segundo mecanismo podría estar asociado con transporte de aniones, con el fin de mantener la electro-neutralidad del agua de mar vacuolizada.

Desafíos asociados a proxies de foraminíferos: Segunda parte

La composición de elementos traza y menores e isótopos en conchas de foraminíferos varía no sólo en función de cambios en el entorno de calcificación, sino también como respuesta a procesos fisiológicos complejos. Estos últimos son conocidos como "el efecto vital", el cual hace referencia a procesos biológicamente controlados, los cuales son responsables de la variabilidad adicional observada en la incorporación de elementos traza y menores en conchas de foraminíferos (Nürnberg et Al., 1996a; Erez, 2003, Eggins et Al., 2003; Sadekov et Al., 2005; Bentov y Erez, 2006). El objetivo de **Capítulo 5** es determinar la magnitud y los mecanismos detrás de los efectos ontogenético e inter-individual (entre individuos de la misma especie) en la incorporación de Mg y Sr en conchas del foraminífero planctónico *Globigerinoides sacculifer*. Este estudio se llevó a cabo bajo condiciones de laboratorio controladas. Aunque la temperatura es el parámetro dominante que controla la proporción Mg/Ca en las conchas de este foraminífero, se observó que la variabilidad inter-

individual en la proporción Mg/Ca contribuye en 2-3°C a la variación aparente en $T_{Mg/Ca}$. La variabilidad debida a procesos ontogenéticos es responsable de una disminución de aproximadamente 1°C en $T_{Mg/Ca}$ por cada cámara construida (de la más antigua a la más reciente). Este efecto ontogenético es significativo y puede influir en las reconstrucciones de temperatura basadas en Mg/Ca de foraminíferos. Por lo tanto, en este capítulo se propuso una nueva fórmula experimental que estima la proporción de Mg/Ca con relación a la temperatura y al diámetro máximo de la concha (MSD), a saber: $Mg/Ca=(0.55-0.0001*MSD)e^{0.089*T}$. En el **Capítulo 6**, se analiza la composición química de las conchas calcíticas de foraminíferos bentónicos de profundidad (*Cibicidoides pachyderma*). Estos organismos fueron recolectados de muestras superficiales de núcleos sedimentarios provenientes de una profundidad de 550 m en la Bahía de Vizcaya, costa noroccidental francesa sobre el Océano Atlántico. Los cambios en la química del agua de fondo y el agua intersticial se reflejan en variaciones inter e intra-individuales (entre cámaras del mismo individuo), de los elementos traza y menores de las conchas analizadas. La incorporación de bario (Ba) y manganeso (Mn) en las conchas calcíticas de los foraminíferos indican su migración vertical dentro de los centímetros más superficiales del sedimento. La ausencia de variabilidad inter e intra-individual en Sr/Ca evidencia que, aparentemente, este elemento no es afectado por la química del agua, ni por patrones de migración u otros efectos vitales (procesos fisiológicos). Los cambios observados en las concentraciones de Ba y Mn en el agua intersticial se vieron reflejados en la composición química de las conchas calcíticas de estos foraminíferos bentónicos.

Investigaciones futuras en foraminíferos

El incremento en las emisiones de CO_2 ocasiona cambios en la composición química de los océanos. Uno de los cambios más importantes involucra la disminución del pH en el agua de mar (Zeebe and Wolf-Gladrow, 2005). Estas variaciones en la composición química de los océanos pueden tener consecuencias significativas en los procesos de biomineralización de organismos que construyen conchas calcáreas, como los foraminíferos. Así, un incremento marcado de CO_2 en la atmósfera aumenta el contenido de CO_2 en los océanos y como consecuencia, se produce una disminución en el estado de saturación de la calcita, Ω, en la capa superficial del agua de mar. Cambios en la concentración de carbonatos del agua de mar ($[CO_3^{2-}]$) afectan en mayor proporción los procesos de calcificación en foraminíferos que variaciones de Ω

(**Capítulos 2** y **4**). Bajo condiciones elevadas de CO_2 la tasa de formación de calcita disminuye, siendo esta respuesta más pronunciada en foraminíferos planctónicos que en especies bentónicas de aguas someras (Kısakürek et al., 2008; Bijma et al., 1999; **Capítulos 2 y 3** de esta tesis). Debido a que los foraminíferos usan tanto el carbonato como el bicarbonato del agua de mar en la construcción de nuevas cámaras, el proceso de acidificación del océano no tendría por qué ocasionar un efecto adverso en la capacidad de los foraminíferos para calcificar sus conchas. Si se entiende que como consecuencia de la acidificación del océano se produce un incremento del carbono inorgánico disuelto (CID), se esperaría entonces una mayor cantidad de carbono disponible para dichos procesos de calcificación. Sin embargo, la conversión de bicarbonato en carbonato – usado durante la producción de nueva calcita- requiere una inversión energética más elevada por parte del individuo. Las limitaciones de los foraminíferos al momento de invertir más energía en los procesos de calcificación fueron demostradas en el **Capítulo 2**. Es así como la habilidad de estos organismos para adaptarse a la continua acidificación de los océanos resulta bastante difícil de predecir *a priori*.

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Curriculum Vitae

Adriana Dueñas Bohóquez was born in Bogotá D.C., Colombia, on the 27th of December 1975. After she completed her secondary education in 1994, she studied Biology at the National University of Colombia (Universidad Nacional de Colombia) in Bogotá, D.C. She did her bachelor thesis on Marine Chemistry Ecology of Coral Reef Sponges from the Caribbean Coast of Colombia under the supervision of Dr. Sven Zea. She graduated with honors in 2000. Before continuing with her Master studies, she spent two years working and travelling in England, Spain, Italy and The Netherlands. In 2002, she worked as an intern in the Marine Invertebrate Section at the Natural History Museum in London, England. At the end of 2002 and for the following two years, she did her Master studies at the University of Barcelona, Spain. During this time, Adriana worked on Marine Pollution caused by heavy metals in Seagrass Ecosystems of the Mediterranean Sea. She finished her master thesis at the end of 2004 and moved to Utrecht, The Netherlands. At the beginning of 2005, she began to work as an intern at the Faculty of Geosciences in Utrecht University and at the end of summer in the same year, she started her PhD studies at the Geochemistry Department of the Faculty of Geosciences, Utrecht University. Her PhD research was supervised by Dr. Gert-Jan Reichart, Prof. Bert van der Zwaan and Prof. Gert de Lange. This PhD project was funded by the Dutch Organization for Scientific Research (NWO), under project PaleoSalt, and the European Science Foundation (ESF) under the EUROCORES Programme, EuroCLIMATE.

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Appendix A. Chapter 2.

Table A1. DIC values obtained by two methods.

Controls (Salinity)	DIC[a] Analyzer	SD[b]	DIC CO_2sys[a] program	SD[b]	RSD (%)[c]
30	1772	2	1702	28	1.4
36	2099	10	2053	36	0.8
39	2191	4	2216	6	0.4

[a]Values in µmol/kg.
[b]Standard deviation.
[c]Relative Standard Deviation = absolute value of the coefficient of variation expressed as a percentage. It quantifies the precision of the measurements (a percentage lower than 3% indicates an acceptable precision).

Table A2. Mg and Sr concentrations in ppm of the calcite crystal (Icelandspar) obtained by LA-ICP-MS and ICP-AES.

Elements	LA-ICP-MS[a]	SD[b]	ICP-AES[c]	SD[b]
Mg	674	57	656	62
Sr	184	15	160	8

[a]Data calculated from 643 analyses taken during a 4 year period.
[b]Standard deviation.
[c]Data calculated from 10 analyses.

Table A3. Mg/Ca of cultured foraminifera shells (Chambers stage 19; control culture experiments. Data shown in Figure 2B).

Experiment No.	Salinity	Specimen No.	Mg/Ca (mmol/mol)	Mg/Ca (mmol/mol)[a]	Average Mg/Ca (mmol/mol)
1 (control 1)	30	1	3.54 ± 0.34		
			3.33 ± 0.10	3.43 ± 0.24	
		2	3.22 ± 0.10		
			2.95 ± 0.14	3.08 ± 0.19	
		3	3.29 ± 0.07		
			2.64 ± 0.13	2.97 ± 0.39	3.16 ± 0.24
5 (control 2)	36	1	3.53 ± 0.12	3.51 ± 0.37	
			3.89 ± 0.14		
			3.10 ± 0.20		
		2	3.99 ± 0.08		
			4.24 ± 0.03	4.12 ± 0.15	
		3	5.16 ± 0.27	5.16 ± 0.27	
		4	4.26 ± 0.07		
			3.93 ± 0.08	4.09 ± 0.20	
		5	3.06 ± 0.01		
			2.81 ± 0.07	2.94 ± 0.15	
		6	4.78 ± 0.04		
			4.44 ± 0.13	4.61 ± 0.27	
		7	2.43 ± 0.19		
			2.75 ± 0.12	2.59 ± 0.23	
		8	3.29 ± 0.10		
			3.47 ± 0.09	3.38 ± 0.13	3.80 ± 0.86
9 (control 3)	39	1	3.22 ± 0.11	3.22 ± 0.11	
		2	4.14 ± 0.31	4.14 ± 0.31	
		3	5.57 ± 0.16	5.57 ± 0.16	
		4	5.31 ± 0.47	5.31 ± 0.47	4.56 ± 0.96

Values are given with ± standard deviations.
[a]Values correspond to one laser ablation (LA) analysis or the average of two to three LA analyses.

Table A4. Mg/Ca of cultured foraminifera shells (Chambers stage 18, all culture experiments)[a].

Experiment No.	Salinity	Specimen No.	Mg/Ca (mmol/mol)	Mg/Ca (mmol/mol)[b]	Average Mg/Ca (mmol/mol)
1 (control 1)	30	1	4.45 ± 0.13		
			4.06 ± 0.02	4.25 ± 0.24	
		2	5.72 ± 0.11		
			5.86 ± 0.31	5.79 ± 0.20	
		3	3.14 ± 0.26		
			3.27 ± 0.54	3.21 ± 0.36	4.42 ± 1.30
2	36	1	3.71 ± 0.03	3.71 ± 0.03	
		2	5.14 ± 0.23	5.14 ± 0.23	4.42 ± 1.01
3	39	1	6.71 ± 0.51	6.71 ± 0.51	
		2	6.69 ± 0.72	6.69 ± 0.72	
		3	4.24 ± 0.67	4.24 ± 0.67	
		4	4.81 ± 0.40	4.81 ± 0.40	5.61 ± 1.28
5 (control 2)	36	1	5.20 ± 0.01		
			5.22 ± 0.28	5.21 ± 0.16	
		2	5.65 ± 0.15	5.65 ± 0.15	
		3	5.19 ± 0.03		
			5.24 ± 0.06	5.21 ± 0.05	
		4	4.09 ± 0.02		
			4.44 ± 0.13	4.27 ± 0.21	
		5	5.97 ± 0.01	5.97 ± 0.01	
		6	4.79 ± 0.17		
			4.45 ± 0.01	4.62 ± 0.22	5.16 ± 0.63
6	39	1	6.28 ± 0.05		
			6.25 ± 0.32		
			6.16 ± 0.51	6.23 ± 0.27	
		2	5.48 ± 0.20		
			5.51 ± 0.27	5.50 ± 0.20	5.86 ± 0.52
7	30	1	4.13 ± 0.21	4.13 ± 0.21	
		2	2.57 ± 0.14		
			3.17 ± 0.18	2.87 ± 0.11	3.50 ± 0.89
8	36	1	4.64 ± 0.27	4.64 ± 0.27	
		2	4.16 ± 0.19	4.16 ± 0.19	4.40 ± 0.34
9 (control 3)	39	1	3.53 ± 0.05		
			3.28 ± 0.35	3.41 ± 0.25	
		2	3.97 ± 0.27	3.97 ± 0.27	
		3	6.10 ± 0.21	6.10 ± 0.21	
		4	6.53 ± 0.20	6.53 ± 0.20	
		5	5.08 ± 0.41		
			5.16 ± 0.19	5.12 ± 0.27	5.03 ± 1.34

Values are given with ± standard deviations.
[a]Experiment No. 4 is not reported due to lack of specimens.
[b]Values correspond to one laser ablation (LA) analysis or the average of two to three LA analyses.

Table A5. Sr/Ca of cultured foraminifera shells (Chambers stage 18, all culture experiments)[a].

Experiment No.	Salinity	Specimen No.	Sr/Ca (mmol/mol)	Sr/Ca (mmol/mol)[b]	Average Sr/Ca (mmol/mol)
1 (control 1)	30	1	1.46 ± 0.11		
			1.22 ± 0.09	1.34 ± 0.17	
		2	1.38 ± 0.16		
			1.33 ± 0.15	1.36 ± 0.04	
		3	1.20 ± 0.15		
			1.32 ± 0.17	1.26 ± 0.08	1.32 ± 0.05
2	36	1	1.23 ± 0.18	1.23 ± 0.18	
		2	1.37 ± 0.24	1.37 ± 0.24	1.30 ± 0.10
3	39	1	1.64 ± 0.33	1.64 ± 0.33	
		2	1.45 ± 0.30	1.45 ± 0.30	
		3	1.37 ± 0.07	1.37 ± 0.07	
		4	1.29 ± 0.07	1.29 ± 0.07	1.44 ± 0.15
5 (control 2)	36	1	1.40 ± 0.09		
			1.43 ± 0.09	1.42 ± 0.02	
		2	1.29 ± 0.06		
			1.38 ± 0.07	1.34 ± 0.06	
		3	1.35 ± 0.05		
			1.37 ± 0.06	1.36 ± 0.01	
		4	1.29 ± 0.09		
			1.34 ± 0.10	1.32 ± 0.04	
		5	1.29 ± 0.07	1.29 ± 0.07	
		6	1.27 ± 0.07	1.27 ± 0.07	
		7	1.50 ± 0.08		
			1.42 ± 0.08	1.46 ± 0.06	1.35 ± 0.07
6	39	1	1.41 ± 0.12		
			1.39 ± 0.12		
			1.45 ± 0.13	1.42 ± 0.03	
		2	1.46 ± 0.08		
			1.34 ± 0.07	1.40 ± 0.08	1.41 ± 0.01
7	30	1	1.41 ± 0.08	1.41 ± 0.08	
		2	1.30 ± 0.08		
			1.41 ± 0.09	1.36 ± 0.08	1.38 ± 0.04
8	36	1	1.40 ± 0.10	1.40 ± 0.10	
		2	1.24 ± 0.10	1.24 ± 0.10	1.32 ± 0.11
9 (control 3)	39	1	1.19 ± 0.09		
			1.26 ± 0.09	1.23 ± 0.05	
		2	1.30 ± 0.11	1.30 ± 0.11	
		3	1.41 ± 0.13	1.41 ± 0.13	
		4	1.42 ± 0.21		
			1.46 ± 0.21	1.44 ± 0.03	1.34 ± 0.10

Values are given with ± standard deviations.
[a]Experiment No. 4 is not reported due to lack of analysis.
[b]Values correspond to one laser ablation (LA) analysis or the average of two to three LA analyses.

Table A6. Mg/Ca and Sr/Ca of cultured foraminifera shells (Chamber stage 18, data shown in Figures 5A, B, C and D).

Ω [a]	$[CO_3^{2-}]$ (µmol/kg)	Specimen No.	Mg/Ca (mmol/mol)	Average Mg/Ca (mmol/mol)	Sr/Ca (mmol/mol)	Average Mg/Ca (mmol/mol)
3.16	133	1	5.82 ± 0.33	5.82 ± 0.33	1.41 ± 0.11	1.41 ± 0.11
10.16	426	1	6.76 ± 0.32		1.66 ± 0.07	
		2	6.67 ± 0.26		1.62 ± 0.04	
		3	4.12 ± 0.39		1.39 ± 0.03	
		4	5.56 ± 0.21		1.58 ± 0.06	
		5	6.94 ± 0.49	6.01 ± 1.19	1.63 ± 0.06	1.58 ± 0.11
13.09	550	1	4.80 ± 0.44	4.80 ± 0.44	1.54 ± 0.11	1.54 ± 0.11

Values are given with ± standard deviations.
[a]From da Rocha et al. in prep.

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Appendix B. Chapter 4.

Table B1. Mg/Ca and Sr/Ca ratios of *Ammonia tepida*.

Experiment No.	Specimen No.	Mg/Ca (mmol/mol)	Sr/Ca (mmol/mol)
1	1	1.41 ± 0.13	1.37 ± 0.11
	2	1.78 ± 0.17	1.49 ± 0.12
	3	1.38 ± 0.13	1.47 ± 0.12
	4	1.39 ± 0.14	1.39 ± 0.11
	5	1.88 ± 0.19	1.46 ± 0.12
	6	0.84 ± 0.09	1.61 ± 0.14
	7	1.60 ± 0.17	1.53 ± 0.13
	8	1.95 ± 0.21	1.61 ± 0.14
	9	2.48 ± 0.27	1.31 ± 0.12
2	1	1.56 ± 0.07	1.21 ± 0.06
	2	1.29 ± 0.05	1.26 ± 0.05
	3	1.26 ± 0.06	1.30 ± 0.07
	4	1.42 ± 0.07	1.41 ± 0.08
	5	1.67 ± 0.07	1.30 ± 0.06
	6	1.26 ± 0.05	1.41 ± 0.07
	7	2.25 ± 0.10	1.36 ± 0.08
	8	2.78 ± 0.13	1.53 ± 0.09
3	1	1.53 ± 0.18	1.32 ± 0.13
	2	1.43 ± 0.17	1.43 ± 0.15
	3	1.14 ± 0.14	1.45 ± 0.15
	4	1.33 ± 0.17	1.34 ± 0.14
	5	1.77 ± 0.23	1.46 ± 0.16
	6	2.87 ± 0.38	1.26 ± 0.14
	7	1.27 ± 0.17	1.55 ± 0.18
	8	2.11 ± 0.30	1.39 ± 0.16
	9	3.01 ± 0.42	1.34 ± 0.16
	10	2.29 ± 0.32	1.35 ± 0.16
4	1	1.08 ± 0.17	1.71 ± 0.22
	2	2.79 ± 0.45	1.36 ± 0.18
	3	1.26 ± 0.21	1.48 ± 0.20
	4	2.60 ± 0.43	1.47 ± 0.20
	5	3.02 ± 0.17	1.33 ± 0.08
	6	1.69 ± 0.10	151 ± 0.09
	7	1.63 ± 0.11	1.62 ± 0.10
	8	2.80 ± 0.18	1.43 ± 0.09
	9	1.97 ± 0.13	1.,38 ± 0.10
	10	1.85 ± 0.13	1.41 ± 0.11
	11	1.27 ± 0.11	1.87 ± 0.16

Values are given with ± standard deviations.

Table B2. Mg/Ca and Sr/Ca ratios of *Heterostegina depressa*.

Experiment No.	Specimen No.	Mg/Ca (mmol/mol)	Sr/Ca (mmol/mol)
1	1	140.40 ± 5	2.63 ± 0.10
	2	126.12 ± 8	2.63 ± 0.18
	3	150.11 ± 14	2.59 ± 0.26
	4	117.00 ± 11	2.32 ± 0.24
	5	130.30 ± 7	2.35 ± 0.14
	6	140.00 ± 7	2.73 ±0.16
	7	128.00 ± 8	2.53 ± 0.18
	8	141.30 ± 19	2.37 ± 0.31
	9	138.50 ± 12	2.26 ± 0.22
2	1	132.00 ± 15	2.40 ± 0.39
	2	143.31 ± 17	2.48 ± 0.41
	3	140.14 ± 4	2.94 ± 0.10
	4	125.10 ± 5	2.56 ± 0.11
	5	122.00 ± 4	2.21 ±0.08
	6	131.00 ± 5	2.72 ± 0.11
	7	124.00 ± 4	2.50 ± 0.11
	8	122.00 ± 5	2.60 ± 0.12
	9	143.50 ± 8	2.64 ± 0.18
	10	140.00 ± 8	2.79 ± 0.20
	11	129.00 ± 6	2.33 ± 0.14
3	1	140.10 ± 13	2.53 ± 0.26
	2	135.00 ± 9	2.19 ± 0.16
	3	126.00 ± 6	2.29 ± 0.10
	4	147.30 ± 9	2.24 ± 0.13
	5	134.00 ± 9	2.58 ± 0.15
	6	127.00 ± 7	2.80 ± 0.13
	7	134.00 ± 9	2.34 ± 0.14
	8	119.40 ± 7	2.56 ± 0.13
	9	134.11 ± 8	2.43 ± 0.12
	10	134.00 ± 7	2.24 ± 0.11
	11	135.34 ± 9	2.24 ± 0.13
	12	144.34 ± 10	2.64 ± 0.17
4	1	146.20 ±19	2.26 ± 0.26
	2	145.12 ± 10	2.80 ± 0.17
	3	157.30 ± 11	2.63 ± 0.16
	4	134.43 ± 12	2.19 ± 0.17
	5	135.12 ± 16	2.82 ± 0.28

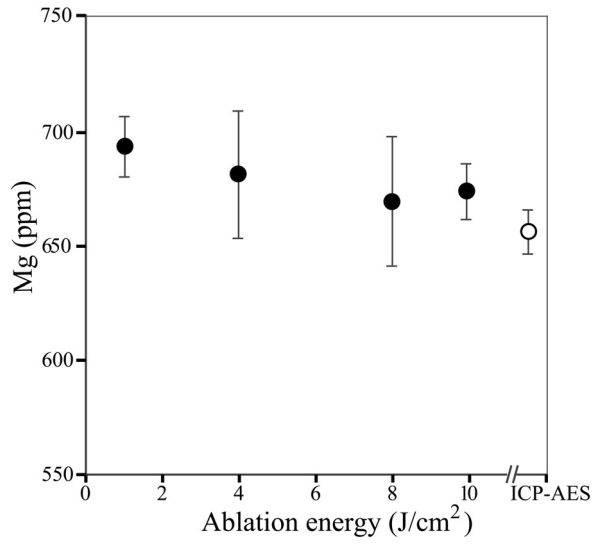Values are given with ± standard deviations.

Figure B1. Mg (average ^{24}Mg and ^{26}Mg) concentration in the calcite crystal Icelandspar vs. ablation energy.

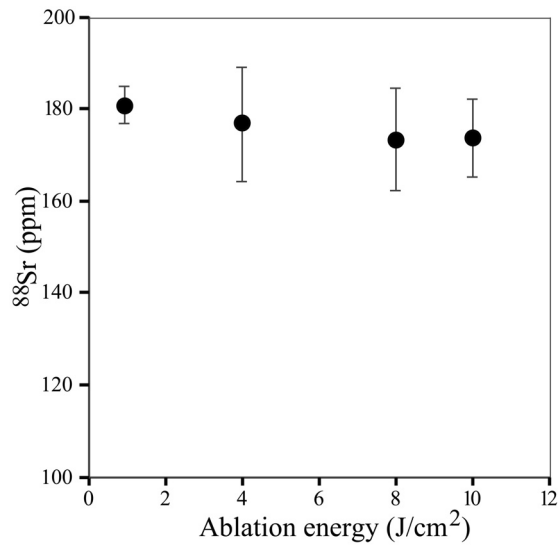Figure B2. ^{88}Sr concentration in the calcite crystal Icelandspar vs. ablation energy.

Appendix C. Chapter 5.

Table C1. DIC values obtained by two methods.

Salinity	DIC Analyser (µmol/kg SW)	DIC CO_2sys program (µmol/kg SW)	SD[a]	Rel. STDV (%)
36	2099	2053	16	0.8

[a]standard deviation.

Table C2. Mg and ^{88}Sr concentrations in ppm of the calcite crystal (Icelandspar) obtained at different ablation energy densities.

Energy density (J/cm^2)	Mg[a]	SD[b]	^{88}Sr	SD[b]
1	692	16	182	4
4	685	30	178	13
8	664	32	176	10
10	676	10	176	5

[a]Mg values correspond to the average value of ^{24}Mg and ^{26}Mg.
[b]Standard deviation.

Table C3. Mg and Sr concentrations in ppm of the calcite crystal (Icelandspar) obtained by LA-ICP-MS and ICP-AES.

Elements	LA-ICP-MS[a]	SD[b]	ICP-AES[c]	Standard deviation
Mg	674	57	656	62
Sr	184	15	160	8

[a]Data calculated from 643 analyses.
[b]Dtandard deviation.
[c]Data calculated from 10 analyses.
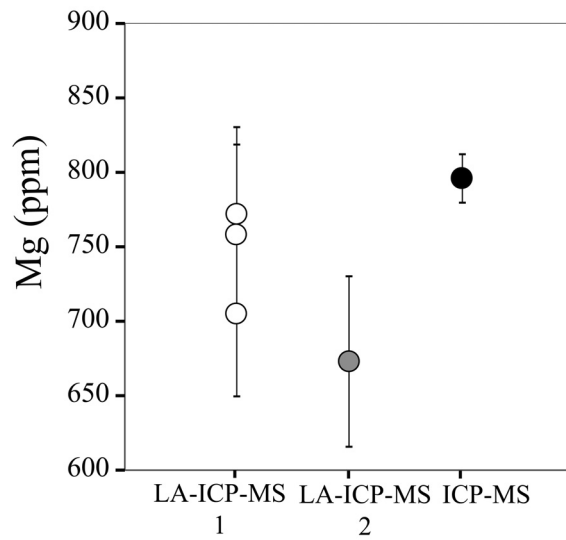
Appendix D. Chapter 6.

Figure D1. Mg measurements of the calcite crystal Icelandspar: LA-ICP-MS 1 corresponds to laser-ablation measurements performed during the analyses of tests of *Cibicidoides pachyderma*; LA-ICP-MS 2 corresponds to the laser-ablation analyses performed during a four-year period (n=643); ICP-MS corresponds to inductively coupled plasma mass spectrometry analyses.