



**Elements and Isotopes  
in Foraminifera**

Magnesium Uptake, Biomineralization  
and Proxy Application

Linda K. Dämmer

# **ELEMENTS AND ISOTOPES IN FORAMINIFERA**

MAGNESIUM UPTAKE,  
BIOMINERALIZATION  
AND PROXY  
APPLICATION

BY LINDA K. DÄMMER

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# **Elements and Isotopes in Foraminifera**

## **Magnesium Uptake, Biomineralization and Proxy Application**

### **Elementen en Isotopen in foraminiferen**

### **Magnesium inbouw, biomineralizatie en toepassen van proxies**

(met een samenvatting in het Nederlands)

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## **Linda Karoline Dämmer**

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# SAMENVATTING IN HET NEDERLANDS

Het klimaat op aarde is snel aan het veranderen en dat wordt grotendeels veroorzaakt door uitstoot van broeikasgassen. Deze (op geologische schaal) vrij snelle stijging van CO<sub>2</sub> in de atmosfeer leidt tot opwarming, maar heeft ook gevolgen voor andere aspecten van het klimaat op aarde, zoals bijvoorbeeld de hydrologische cyclus en de zuurgraad van de oceanen. Reconstructie van klimaatverandering in het verleden is een belangrijke manier om te kunnen voorspellen hoe het klimaatsysteem op aarde op de toename van broeikasgassen gaat reageren. Gedurende de geologische geschiedenis heeft een diversiteit van klimaat-toestanden op aarde geheerst, van een planeet die volledig bedekt was met ijs (de zogenaamde 'sneeuwbal aarde') tot verschillende thermische maxima, met (sub)tropische temperaturen in het Noordpoolgebied. Het begrijpen van de onderliggende mechanismen die verantwoordelijk waren voor deze grote en soms snelle schommelingen is het onderwerp van paleoklimatologie en aangezien de methoden voor het bestuderen van omstandigheden en het klimaat van vroeger zich vaak concentreren op sporen die in oceanen zijn afgezet, overlapt dit veld grotendeels met paleoceanografie. Het is het belangrijkste doel van paleoceanografie is om klimaattransities in het verleden te bestuderen en te begrijpen welke mechanismen verantwoordelijk zijn voor de veranderingen in temperatuur, zoutgehalte, opgeloste zuurstofconcentraties, oceaan circulatie, zuurgraad, enz.

Honderden miljoenen jaren lang hebben mariene, kalkvormende organismen schelpen en skeletten geproduceerd die zich in de zeebodem hebben opgehoopt. De chemische samenstelling van deze fossielen weerspiegelen de omstandigheden (temperatuur, zoutgehalte, pH, enz.) waarin de organismen leefden. Dankzij hun wijdverbreide aanwezigheid in alle oceanen, het gemak waarmee ze fossiliseren en hun lange geologische geschiedenis zijn schelpen van foraminiferen een populair hulpmiddel in de paleoceanografie. Deze foraminiferen zijn ééncellige protisten, waarvan sommigen een schelp van organisch materiaal bouwen, maar er zijn ook groepen die kalk maken; dat wil zeggen, ze maken een schelpje dat bestaat uit calciumcarbonaat (CaCO<sub>3</sub>). Ze maken hun schaalpjes door regelmatig kamers toe te voegen, die in essentie uit een wand van kalk bestaan waarbinnen een holle ruimte overblijft en de groeiende cel zich kan huizen.



Het aldus geproduceerde calciumcarbonaat bevat ook lage concentraties van andere elementen, waaronder magnesium (Mg), waarvan de hoeveelheid die wordt ingebouwd afhankelijk is van de temperatuur van het zeewater. Bij hogere temperaturen nemen foraminiferen meer Mg op, zodat een kalibratie van deze relatie gebruikt kan worden om met het Mg-gehalte van fossiele schelpen (uitgedrukt als Mg/Ca) temperaturen uit het verleden te reconstrueren. Omdat sommige foraminiferen in de waterkolom leven (‘planktonische’ soorten) en andere op de zeebodem (‘benthische’ soorten), kan de combinatie van Mg/Ca uit deze twee groepen worden gebruikt om verticale temperatuurgradiënten in vroegere oceanen te bestuderen. Daarmee kan oceaancirculatie in het verleden beter begrepen worden, wat van belang kan zijn om bijvoorbeeld veranderingen voorspellen als gevolg van de huidige afremming in de circulatie in de noordelijke Atlantische Oceaan door het alsmaar voortdurende smelten van land- en zee-ijs.

Het gebruik van de chemische samenstelling van kalk van foraminiferen om condities in het verleden te bestuderen, zijn echter niet perfect. Vaak hebben meerdere omgevingsparameters een effect op de inbouw van een element of de fractionering van een isotoop. In het geval van de inbouw van magnesium, hebben naast temperatuur ook het zoutgehalte van het zeewater, de pH en de concentratie van Mg in zeewater elk een invloed op de Mg/Ca van het kalk van foraminiferen. Bovendien verschilt de relatie tussen Mg/Ca en temperatuur van soort tot soort en zelfs binnen een soort of binnen een enkel individu kan de Mg/Ca (ook als de condities gelijk zijn) variëren. Met de toegenomen resolutie waarmee elementen binnen een enkele foraminifeer kunnen worden bestudeerd, is inmiddels ook aangetoond dat zelfs binnen één enkele kamerwand de Mg/Ca sterk kan variëren.

In dit proefschrift laat ik zien dat licht ook de opname van Mg beïnvloedt. Foraminiferen die meer uren daglicht ervaren in een periode van 24 uur, hebben gemiddeld een lagere Mg/Ca vergeleken met diegenen die een langere tijd in het donker doorbrengen. Het verschil tussen Mg/Ca in kalk dat overdag en 's nachts wordt gevormd kan zowel de timing van kamerbouw in foraminiferen kunnen verklaren alsmede de variabiliteit in

de chemische samenstelling van hun schelpjes. Een foraminifeer die aan het einde van de dag met zijn kamervorming begint, zal kalk maken met een hogere Mg/Ca dan eentje die bij zonsopgang begint, los van de temperatuur van het zeewater waarin ze leven. Waarom licht precies de opname van Mg beïnvloedt, moet is ontdekt worden: eigenlijk zijn de cellulaire processen verantwoordelijk voor de opname van Mg tijdens kamervorming nog niet volledig bekend en vormen een belangrijke motivatie voor het onderzoek van een groot gedeelte van dit proefschrift.

Eén manier om ons begrip van de mechanismen die verantwoordelijk zijn voor de opname van Mg in het kalk van foraminiferen en het effect van de temperatuur daarop te verbeteren, is een onderzoek naar de Mg-isotopen samenstelling ( $\delta^{26}\text{Mg}$ ). Magnesiumionen kunnen drie massa's hebben, afhankelijk van het aantal neutronen in de atoomkern. De meeste atomen wegen 24 massa-eenheden, terwijl een minderheid 25 of 26 eenheden weegt. Als de verhouding tussen deze massa's in een medium verschilt van die in een ander medium, is er sprake van isotopenfractionering tussen deze twee media. Een reeks experimenten met incubatie van foraminiferen bij verschillende temperaturen laat zien dat zij fractioneren tegen de zwaardere isotopen (oftewel ten gunste van de lichtere isotopen) en dat deze fractionering afhankelijk is van de temperatuur. Op basis van deze resultaten heb ik een tweestaps-model ontwikkeld voor Mg-opname in het kalk van de tropische foraminifeer *Amphistegina lessonii*, dat ook op andere soorten kan worden toegepast. De basis van dit model is dat Mg-fractionering plaatsvindt tijdens transport van  $\text{Mg}^{2+}$  vanuit zeewater, door een celmembraan, naar de plaats van calcificatie. Een tweede fractioneringsstap treedt op tijdens precipitatie van kalk vanuit het medium dat de foraminifeer creëert in een microscopische ruimte en die goed geschiedt wordt van het omringende zeewater. Samen beschrijven deze stappen de algehele fractionering tussen Mg-isotopen in zeewater en die in het kalk van de foraminiferen. Het model kan zowel de waargenomen trends in  $\delta^{26}\text{Mg}$  verklaren als het al eerder beschreven fenomeen van laagjes afwisselend hoge en lage Mg/Ca binnen een kamerwand. Dit is namelijk mogelijk omdat het model ongefractioneerd Mg in zeewater kan bevatten aan het begin van de kamervorming. Omdat de uiteindelijke hoeveel-

heid magnesium die wordt ingebouwd ook door dezelfde stappen wordt beïnvloed, kan dit model verklaren waarom die foraminiferensoorten met hogere Mg-concentraties  $\delta^{26}\text{Mg}$ -waarden hebben die dichterbij die van zeewater liggen dan die van hun tegenhangers met een laag Mg/Ca-gehalte.

Een andere factor waarvan wordt vermoed dat deze de hoeveelheid Mg die in biologisch gevormd kalk wordt opgenomen, is de snelheid waarmee  $\text{CaCO}_3$  kristallen groeien. Dit is het onderwerp van een aantal experimenten beschreven in dit proefschrift, waar de invloed van  $\text{CO}_2$  op de groeisnelheid van foraminiferen in twee grote, bentische soorten is beschreven. Omdat foraminiferen tot de belangrijkste mariene kalkvormers in de oceanen behoren, is het begrijpen van hun respons op veranderingen in  $\text{CO}_2$ -niveau cruciaal voor het voorspellen van de contributie in toe- of afnemende kalkvorming op de opname-capaciteit van de oceanen voor  $\text{CO}_2$  uit de lucht. Resultaten laten zien dat beide soorten meer kalk vormen onder matig verhoogde  $\text{CO}_2$ -niveaus. Enkel als de kosten die nodig zijn om verzuring door de hogere  $\text{CO}_2$  concentraties te compenseren te hoog worden, verdwijnt het voordeel van de hogere koolstof concentraties. Dit impliceert dat de reactie op toekomstige atmosferische  $\text{CO}_2$ -concentraties misschien geen eenduidig effect zal hebben op de verschillende soorten foraminiferen, wat wel nodig is om de totale productie van kalk te voorspellen en daarmee het effect te berekenen op de capaciteit van de oceanen om  $\text{CO}_2$  uit de lucht op te nemen.

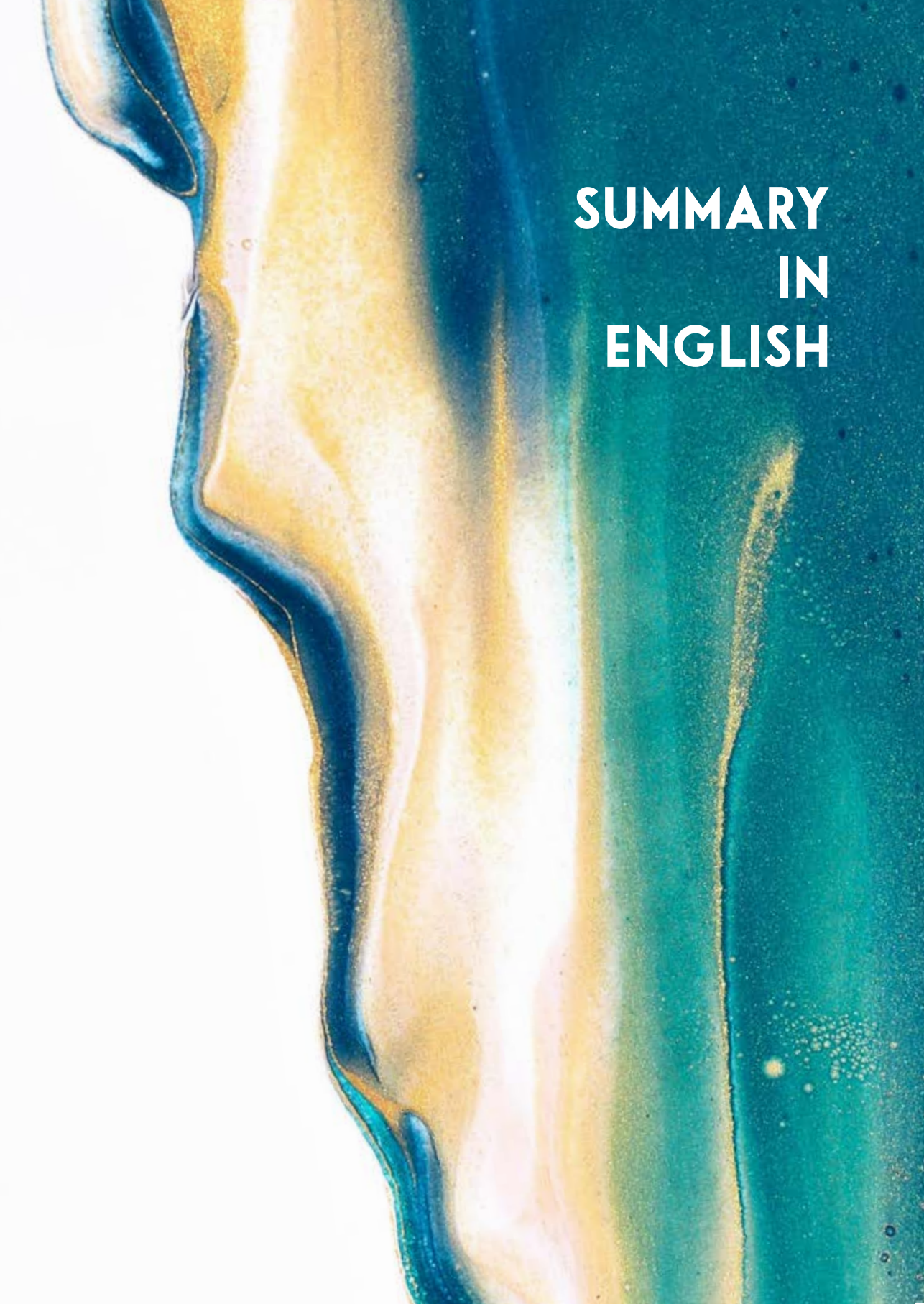
Om observaties in het laboratorium te vertalen naar de chemische samenstelling van foraminiferen in de natuur, heb ik een veldstudie gedaan naar de elementen- en isotopensamenstelling van het kalk van foraminiferen uit de Middellandse Zee. Ik heb planktonische soorten over een Oost-West transect verzameld om de soort-specifieke Mg/Ca-temperatuurkalibraties te verbeteren, met name door lagere temperaturen mee te nemen dan meestal wordt gedaan in zulke kalibratie-studies. Omdat de Mg/Ca-temperatuur correlaties voor veel soorten niet lineair zijn, is het belangrijk om Mg/Ca als temperatuur-reconstructie methode (een zgn. proxy) enkel toe te passen voor temperaturen waarvoor ze zijn gekalibreerd. We hebben ook aangetoond dat het combineren van proxies (Mg/Ca en  $\delta^{18}\text{O}$ ), die kunnen worden gebruikt om het effect van het zoutge-

halte en de temperatuur op beide proxy's te ontlede(n) (en ze dus beiden te reconstrueren), niet noodzakelijkerwijs leidt tot betekenisvolle reconstructies. Mogelijke oorzaken hiervoor zijn temporele en ruimtelijke variaties in beide parameters en het feit dat foraminiferen niet stationair zijn, maar met zeestromingen meebewegen en daarom in de loop van de tijd een variatie in omgevingsparameters ervaren. Dit leidt tot onzekerheden in gereconstrueerde klimaatsignalen en laten dus zien dat het nodig is om de ecologie (bijvoorbeeld levensduur) van foraminiferen beter te kennen.

Dit neemt niet weg dat grotere veranderingen, zoals oceanische circulatiepatronen, kunnen worden gereconstrueerd met behulp van sporenelementen in fossiele foraminiferen. In dit proefschrift beschrijf ik het gebruik van foraminiferen uit een sedimentkern van de Alboránzee om te onderzoeken of signalen van lage zuurstofconcentraties in het oostelijke Middellandse Zeegebied ook te zien zijn in het westelijke gedeelte van de Middellandse Zee in tijden van sapropelvorming – wat een teken zou zijn voor aanzienlijke connectiviteit tussen beide gebieden, ook tijdens de afzetting van de sapropelen in het oostelijke deel. De resultaten geven aan dat toen het oostelijke Middellandse Zeegebied te kampen had met extreem lage zuurstofniveaus, redoxgevoelige elementen zoals mangaan (Mn) in de zeebodem werden gemobiliseerd en vervolgens naar het westen werden verplaatst. Dit blijkt uit de verhoogde Mn concentraties in diverse soorten foraminiferen in die periode, waarbij bentische soorten een grotere aanrijking vertoonden dan planktonische soorten. Daaruit concludeer ik dat het huidige anti-estuariene circulatiepatroon ten tijde van sapropelvorming, hoewel waarschijnlijk langzamer, gewoon in stand bleef.

Ondanks veel open vragen over de mechanismen van kalkvorming door foraminiferen en de integratie van het gebruik van verschillende elementen en isotopen, is hun bruikbaarheid voor paleoceanografische studies en als modelorganismen voor onderzoek naar klimaatverandering, enorm. Dit proefschrift laat zien, dat klimaatreconstructies met behulp van foraminiferen beter kunnen worden als we de ecologie, chemie en biologie van deze ééncelligen beter leren te begrijpen.





# SUMMARY IN ENGLISH

Earth's climate is changing rapidly, which is largely due to the anthropogenic addition of CO<sub>2</sub> to the atmosphere. This (geologically speaking) sudden increase leads to rising global temperatures but also affects other parts of system Earth, including the hydrological cycle and the acidity of the oceans' surface. Reconstructions of past climate behavior is a valuable method to predict how the earth's climate will react to these rapid changes. During geological history, the earth has seen a variety of climates, from a planet completely covered in ice (so-called 'snowball earth') to several thermal maxima, with (sub)tropical temperatures in the Arctic. Understanding the underlying mechanisms that were responsible for these large and sometimes rapid swings is the subject of paleoclimatology and since the methods for studying past conditions and climates often focus on traces that were deposited in ancient oceans, this field overlaps largely with palaeoceanography. It is the main goal of palaeoceanography to study past climate transitions and understand the forces responsible for changes in seawater temperature, salinity, oxygenation, nutrient cycling, currents, etc.

For hundreds of millions of years, marine calcifying organisms have left shells and skeletons in the seabed with a chemical composition reflecting the conditions (temperature, salinity, pH, etc.) in which the organisms were living. Thanks to their widespread occurrence, their high fossilisation potential and their long geological record, fossil shells of foraminifera are a popular tool in palaeoceanography. These foraminifera are single-celled protists, some of which build a shell of organic material, but also include groups that calcify; that is, they make a shell consisting of calcium carbonate (CaCO<sub>3</sub>). They grow these shells by regular addition of chambers during their lifetime. These chambers essentially consist of a chamber wall providing a hollow space in which the cell resides.

The calcium carbonate produced by foraminifera when building their test chambers also contains small amounts of other elements, including Magnesium (Mg), of which the amount that is incorporated depends on seawater temperature. At higher temperatures, foraminifera incorporate more Mg, so that with a calibration of this relationship,

the Mg content of fossil shells (expressed as the Mg/Ca) can be used to reconstruct past temperatures. Since some foraminifera live in the water column ('planktonic' species) and others live at the seafloor ('benthic' species), the combination of Mg/Ca from these two groups can be used to reconstruct the vertical temperature gradient in past oceans, which in turn can help to understand ocean circulation in the past. The latter may be important when, for example, studying the changes in overturning circulation in the northern Atlantic due to the ongoing melting of land- and sea ice.

However, the chemical reconstruction tools based on the chemical composition of foraminiferal calcium carbonate are not perfect. There are usually multiple environmental parameters that affect the incorporation of an element or the fractionation of an isotope. In case of Magnesium incorporation, seawater salinity, pH and the concentration of Mg in seawater all affect the Mg/Ca of the foraminiferal calcite. Moreover, the relationship between Mg/Ca and temperature varies between species and even within a species or within a single individual. With the increasing spatial resolution at which elements can be studied within a single foraminifer, it was also shown that even within a single chamber wall, the Mg/Ca can vary greatly.

In this thesis, I show that exposure to light also influences the incorporation of Mg. These results show that foraminifera who experience more hours of daylight in a 24-hour period have lower average Mg/Ca compared to those who spend more time in the dark. Since the difference between Mg/Ca in carbonate formed during day and during night, the timing of foraminiferal calcification could well explain the variability in shell chemical composition between specimens. A foraminifer that starts chamber formation at the end of the day will precipitate calcite with a higher Mg/Ca than one that starts at dawn, irrespective of the temperature of the seawater. Exactly why light influences incorporation of Mg remains to be investigated: in fact, the cellular process-

es that are responsible for the uptake of Mg during chamber formation are not fully identified yet and are an important motivation for the research of most of the chapters in this thesis.

One way to improve our understanding of the mechanisms responsible for Mg incorporation into foraminiferal calcite and the effect of temperature thereon, is investigating the Mg isotopic composition ( $\delta^{26}\text{Mg}$ ) of the Mg incorporated in the shell calcite. Most of Mg atoms weigh 24 mass units, while a minority weighs 25 or 26 units, depending on the number of neutrons in the atom. If the ratio between these isotopic masses in a medium differs from that in another medium, there is isotopic fractionation between these two media. A set of experiments with incubations of foraminifera at a range of temperatures, showed that foraminifera fractionate against the heavier isotopes (or in favour of the lighter isotopes) and that this fractionation depends on temperature. Based on these results I developed a two-step model for Mg-incorporation into the calcite of the tropical foraminifer *Amphistegina lessonii*, that can also be applied to other species. Key to this model is the concept that Mg fractionation happens while Mg is transported from seawater to the site of calcification through transmembrane transport. A second fractionation step occurs during precipitation in the calcifying fluid and together these steps describe the overall fractionation between Mg isotopes in seawater and those in the foraminiferal calcite. The model this way explains both the observed trends in  $\delta^{26}\text{Mg}$  as well as the long-known phenomenon of bands of high Mg and low Mg carbonate observed in many foraminifera. The latter is possible since the model allows some unfractionated Mg in seawater to be present at the site of calcification at the onset of chamber formation. Since the amount of incorporated magnesium is also affected by the two steps described in the model, I am able to explain why those foraminiferal species with higher Mg-concentrations also have  $\delta^{26}\text{Mg}$  values closer to that of sea water compared to their low-Mg/Ca counterparts.

Another factor that has been suspected to influence the amount of Mg incorporated into biogenic carbonates is crystal growth rate. This is the focus of the experiments described in chapter 2 where the impact of CO<sub>2</sub> on foraminiferal growth rates in two large, benthic foraminiferal species was analysed. Since foraminifera are among the most important marine calcifiers in today's oceans, understanding their resilience or vulnerability to changes in CO<sub>2</sub> levels is crucial to understanding the feedback loops the anthropogenic increase of atmospheric pCO<sub>2</sub> might trigger. The results show that both species grow more carbonate under slightly elevated CO<sub>2</sub> levels and only when the cost to compensate for the lowered pH overcomes the advantage of an increased availability of inorganic carbon species – building blocks needed to form carbonate – there is a reduction in growth. This implies that the response to future atmospheric CO<sub>2</sub> concentrations will have different effects on foraminiferal species, which is important to recognize since the collective production of carbonate shells in the ocean will affect dissolved CO<sub>2</sub> concentrations and thereby the capacity of the ocean to take up atmospheric CO<sub>2</sub>.

To bridge the gap between observations in the laboratory and the behaviour of foraminifera in nature, I conducted a field study to investigate the elemental and isotopic composition of foraminiferal shells along an environmental gradient. I collected planktonic foraminifera across the Mediterranean Sea to improve species-specific Mg/Ca-temperature calibrations by including lowest temperature ranges tolerated by the species analysed. Since for many species the Mg/Ca-temperature correlations are not linear, it is important to only apply proxies within the same parameter range in which they were developed and tested. We also showed that combining proxies (Mg/Ca and  $\delta^{18}O$ ) sometimes used to disentangle the impacts of salinity and temperature on both proxies, does not necessarily result in meaningful reconstructions of salinity. Potential

causes for this are temporal and spatial variations in both parameters and the fact that foraminifera are not stationary but move with the currents and therefore experience variable conditions during their life-time. The parameters measured at the moment of capture or the foraminifer's death and burial are therefore not necessarily identical to the parameters recorded by the incorporated elements and fractionated isotopes.

Large scale changes in an ocean basin, such as overturning circulation patterns can be reconstructed accurately using trace elements incorporated in fossil foraminiferal shells. In chapter 6 I describe the use of foraminifera from a sediment core from the Alborán Sea to investigate whether signals of low oxygen concentrations in the eastern Mediterranean could be traced back also in the western basin during times of sapropel formation – which would provide evidence for significant connectivity between both areas during these events which are generally considered to have only affected the eastern basin. The results indicate that when the eastern Mediterranean suffered from extremely low oxygen levels, redox-sensitive elements such as Manganese (Mn) were mobilized from the sea floor and then moved to the west, because slightly elevated Mn levels can be found back in a variety of foraminiferal species from that time, with benthic species showing higher increases than planktonic species. It can therefore be concluded that the anti-estuarine circulation pattern in place today was still in operation during sapropel times, albeit likely much reduced in intensity.

Despite many open questions regarding foraminiferal biomineralization mechanisms and elemental incorporation, their usefulness in palaeoceanographic applications and as model organisms for climate change studies is immense. Though only when we understand these processes well enough can the proxies be applied with increasing confidence.

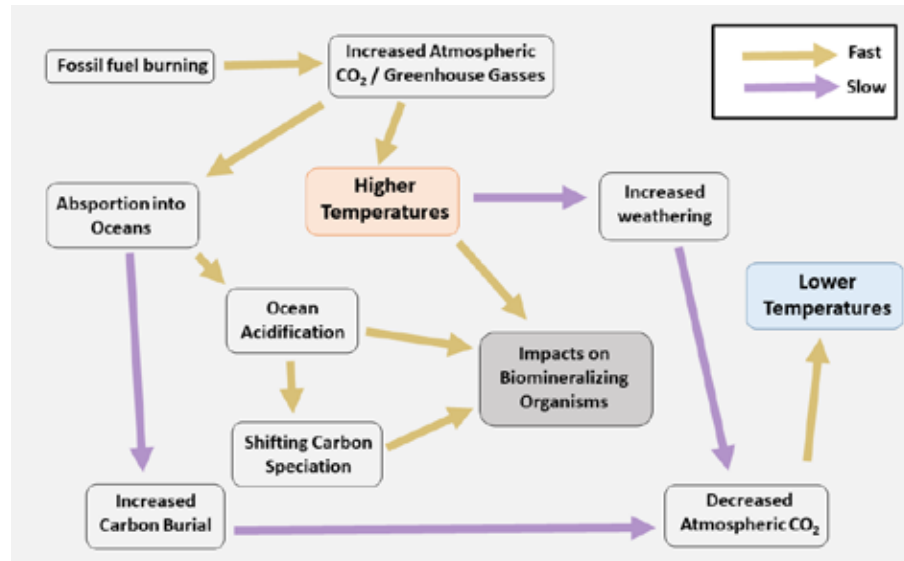




# CHAPTER ONE INTRODUCTION

The unprecedented rate at which greenhouse gasses are emitted into the atmosphere has raised global temperatures by approximately 1.3 °C in the past 150 years (Kaufman et al., 2020; Schuckmann et al., 2023; Siegmund et al., 2019). As a result, glaciers are disappearing and eustatic sea level is rising. At the same time, extreme weather events including droughts and tropical storms will increase in frequency as atmospheric carbon dioxide (i.e., CO<sub>2</sub>) levels continue to rise, causing global warming. Forest fires and desertification are threatening crop yields, which will likely cause famine in some regions (IPCC, 2022) and result in major migrations of the global population in the coming century (Leal Filho et al., 2022). Climate change is also multiplying the effects of pollution and habitat loss on ecosystems as apparent from the dramatic loss in biodiversity around the globe (Talukder et al., 2022). All these changes are happening at an unprecedented rate and will continue to do so unless usage of fossil fuels is soon halted.

So far, the oceans have taken up over 90% of the excess warmth of global warming (Cheng et al., 2020; Schuckmann et al., 2023) and absorbed about 25% of all the CO<sub>2</sub> emitted from fossil fuel burning (Devries, 2022). This has mitigated atmospheric warming, but the additional dissolved carbon dioxide in sea water has been acidifying the upper layers of the ocean. In Earth's history, atmospheric CO<sub>2</sub> levels varied because of the balance between processes emitting CO<sub>2</sub> (e.g., volcanism) and removing CO<sub>2</sub> from the atmosphere (e.g., weathering), which change slowly over geological timescales. In general, it has largely been ocean chemistry and physics that determined atmospheric CO<sub>2</sub>, since the oceans are by far the largest CO<sub>2</sub> reservoir globally (Takahashi and Azevedo, 1982). Ultimately, the earth's feedback loops will also absorb the additional CO<sub>2</sub> added by mankind, but current inputs are much too fast for the natural cycles to equilibrate and compensate timely (Fig. 1). To better understand the natural cycle and ongoing climate change, reconstructions of past seawater chemistry provide a powerful tool.



**Figure 1.** Overview of slow (purple) and fast (yellow) climate change processes and feedback loops.

Studying past changes in temperature and carbon dioxide levels is one of the most important ways to gain understanding of the processes within the climate system and predict the effects of climate change for the coming century (and beyond). Such reconstructions should not only incorporate direct responses but also slow feedbacks in the Earth's system. Past climates are reconstructed to validate and thereby improve climate models that (regionally) predict the rise in temperature for every doubling in  $\text{CO}_2$  level (the so-called climate sensitivity). Especially the response on longer timescales, including the changes in biogeochemical cycles and feedbacks on the global carbon cycle, can only be unravelled when investigating the processes involved on long enough time scales. The past presents us with an archive of a wide range of carbon dioxide levels and temperatures, provided that we develop the tools to reconstruct the relevant parameters from sedimentary or ice records. This essentially places paleoclimate research at the foundation of human climate change preparedness.

When studying past climate, it is important to accurately reconstruct not only environmental parameters (e.g., sea surface temperature or seawater pH) but also the precise timing and rate of change of these parameters over time. Reconstructions can be based on large scale geological features such as changes in the sedimentological record, for instance the appearance of moraines, drop stones or other (de)glaciation deposits.

Fossils provide another valuable source for paleoclimate reconstruction: faunal assemblages for example often change with environmental parameters such as temperature, biological productivity, oxygenation, and salinity. The presence of turtles and other reptile fossils in Antarctic sediments are famous examples showing that the area must have been much warmer when these organisms lived (Tarduno et al., 1998). More precise reconstructions can be based on higher resolution records and/or other approaches. For instance, reconstructions may be based on gases trapped in small bubbles in the layers of ice cores, which can be used to directly measure past atmosphere compositions (e.g., Fischer et al., 1999).

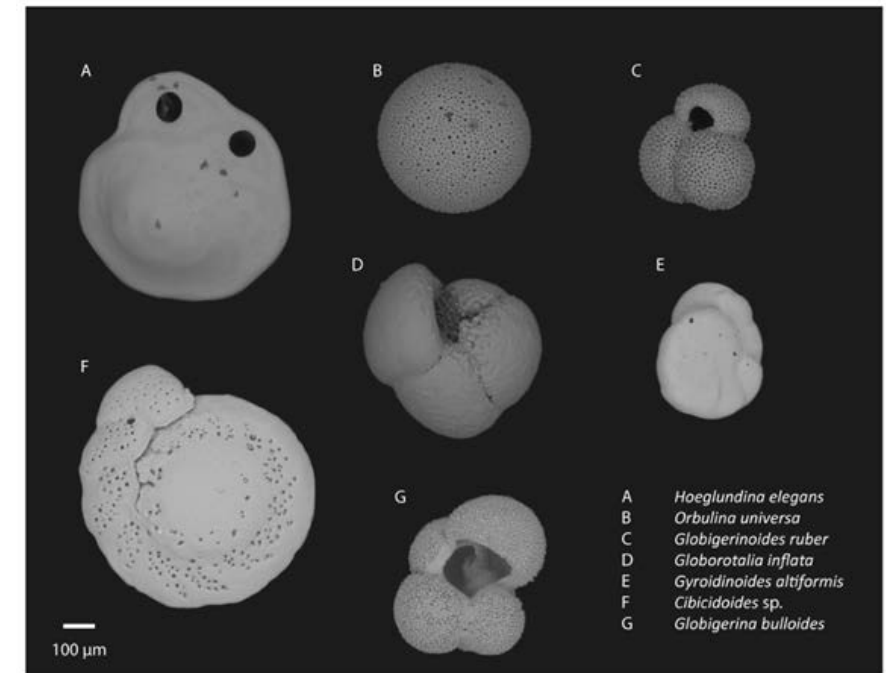
When the target parameter cannot be measured directly, a so-called proxy relationship can be used instead. A proxy is a measurable parameter which changes in a predictable way relative to the target environmental variable. Annual tree rings, with broader rings indicating faster growth, show when years were warmer or wetter than average: in this case the width of a tree ring is a proxy for local temperature and precipitation. Marine calcifying organisms such as corals, molluscs and several groups of plankton can be used similarly and provide important archives for past changes in the ocean. Not only the occurrence of specific species can be used as proxy parameter, also the composition of the organism or fossils themselves can provide detailed information. They form skeletons, spines or shells consisting of the biominerals aragonite or calcite ( $\text{CaCO}_3$ ) containing impurities of many other elements (e.g., Mg and Sr). The concentrations of these elements in the calcium carbonate shells and skeletons vary, partly due to the biological control of the organism on the composition, partly due to the chemical composition of the sea water the organisms precipitate their shell from, and partly due to environmental conditions affecting the uptake of elements (de Nooijer et al., 2014a).

An obvious effect on the amount of an element incorporated is the concentration of that element in the seawater. However, temperature, sea water pH or salinity can also impact the incorporation of many elements in carbonates. For example, higher temperatures increase the amount of magnesium (Mg) incorporated in carbonates (Nürnberg et al., 1996; Rosenthal and Linsley, 2007) and salinity correlates with the concentration of sodium (Na) in some shells (Wit et al., 2013). The amount of an element in biogenic carbonate is commonly expressed relative to the concentration of Ca in the shell: the  $\text{El}/\text{Ca}$  ratio. In case of Mg and Na incorporation, this is thus denoted as  $\text{Mg}/\text{Ca}$  and  $\text{Na}/\text{Ca}$ , respectively.

Like elemental uptake, stable isotope ratios of the elements incorporated into carbonates reflect the environment. Temperature is for instance impacting the relative incorporation of oxygen isotopes (e.g.,  $^{18}\text{O}$  and  $^{16}\text{O}$ ) from sea water into the shell carbonate. This is expressed as the isotopic fractionation and is essentially the difference between the ratio of two isotopes in seawater and that ratio in carbonate and is generally represented by a 'delta-notation' (for example  $\delta^{18}\text{O}$  or  $\delta^{26}\text{Mg}$ ). Higher values always indicates a relative enrichment in the heavier isotope: a smaller or more negative value signifies a relatively higher presence of the lighter isotope in the carbonate (often named 'isotopically depleted'). The stable isotopic composition of the sea water is an important factor here, too.

The organisms used most commonly for such reconstructions are foraminifera (Fig. 2). Foraminifera are single-celled protists, many of which build shells - also called tests - from aragonite or calcite. Especially those foraminifera precipitating calcium carbonate shells are used in this context as not only their occurrence, but also the composition of their carbonate tests can be investigated. The vast majority of all recent and fossil species occur in marine environments, with only few known freshwater species (Siemensma et al., 2017). Their popularity as tools for palaeoceanographic reconstructions is explained by 1) their ubiquitous occurrence throughout virtually all marine habitats, 2) their long geological history (Permian to recent) and 3) their high fossilization potential.

Although many reconstructions based on foraminifera have proven their value in palaeoceanography, there are several issues that limit the precision and accuracy of reconstructions. Particularly planktonic species can have a strong seasonal preference (Thunell et al., 1983) and hence record the conditions only from part of the year, for example when they bloom only during specific seasons. In addition, some planktonic species are suspected to sink or rise in the water column (Field, 2004), therefore experiencing (and recording) a larger range of environmental parameters values, just like they do when they are moved by ocean currents, causing scatter in the resulting data (Steinhardt et al., 2014). These live traits, however, also offer opportunities to investigate seasonality and the thermal structure of the water column and transport via (surface) current can be back tracked to investigate gradients in ocean basins.

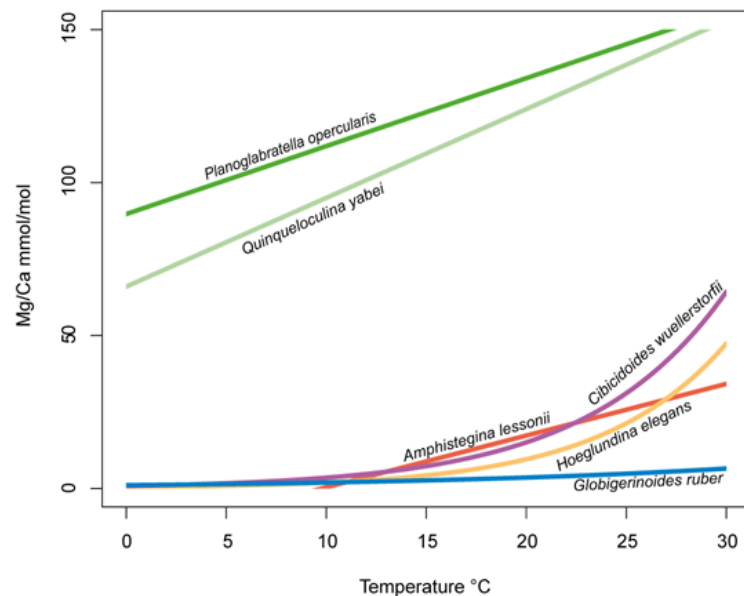


**Figure 2.** A variety of late Pleistocene to Holocene benthic (A, E, F) and planktonic (B, C, D, G) foraminifera from a sediment core from the Iberian margin in the Mediterranean Sea. In *Hoeglundina elegans* (A) laser ablation holes resulting from the trace element measurements of the shell carbonate are visible in the final (F-) and penultimate (F1-) chambers.

The biomineralization process itself also leaves a strong mark on the elemental and isotopic composition of the foraminiferal shell. During their life, most foraminifera grow their shells by sequentially adding chambers. During such an addition, they create a microscopic zone in which foraminifera control the chemistry of the fluid from which they precipitate their calcite. The composition of this fluid is determined by selective transport of ions into and out of this fluid, thereby modulating the relationship between ambient seawater composition and the chemistry of the foraminiferal shell (de Nooijer et al., 2014a). The large variability between species of foraminifera, even when living at identical conditions (de Nooijer et al., 2014b), shows that there are major differences in how strongly foraminifera control the chemistry of the fluid from which they precipitate their calcite. This effect is particularly striking when considering Mg incorporation. Ions of Mg ( $\text{Mg}^{2+}$ ) are very abundant in seawater and inhibit calcite growth. This is likely the reason why many species have evolved specialized mechanisms to reduce the concentration of this ion in the calcifying fluid (Bentov and Erez, 2006; de Nooijer



et al., 2014a). Some foraminiferal species precipitate a calcite with a Mg/Ca similar to that of inorganically precipitated calcite, but many, including all planktonic species, precipitate calcite with approximately 100-fold less Mg in it. Such results show that, irrespective of a mechanistic understanding, application of proxy-relationships to fossil foraminifera are best done by using species-specific calibrations (Fig. 3).



**Figure 3.** Mg/Ca-temperature relationships for a variety of foraminiferal species, using calibrations from van Dijk et al. (2017), Gray et al. (2018), Raitzsch et al. (2008), Reichart et al. (2003) and Toyofuku et al. (2000)

Another type of challenge for accurate reconstructions is the dependency of element incorporation and isotope fractionation on multiple environmental variables. The incorporation of magnesium is clearly affected by temperature (Fig. 3), but salinity and seawater saturation also affect Mg incorporation in many species (Gray et al., 2018; Kısakürek et al., 2008; de Nooijer et al., 2017b). This implies that changes in fossil Mg/Ca will almost always reflect a change in multiple parameters simultaneously. Since this seems the case for most elements and isotopes studied so far and given the observation that not all environmental effects are affecting all elements and isotopes equally, multiple elements should be used whenever possible, to simultaneously reconstruct and disentangle multiple variables in concert.

This thesis consists of five research chapters that aim to understand, improve, and apply foraminifera-based paleoenvironmental proxies. Together, these chapters bridge distinct approaches. Overall, the focus in the thesis shifts from understanding calcification, via proxy calibration and validation to their palaeoceanographic application. The first of these chapters (numbered 2 to 6) investigates the relationship between CO<sub>2</sub> and foraminiferal growth. The results show that the effect of increased CO<sub>2</sub> on foraminiferal growth is not straightforward. Using controlled growth experiments (chapter 3), the effect of a so far understudied parameter, day length, on Mg incorporation is reported while keeping all other parameters constant. Such experiments have the advantage that the effect of a single parameter on incorporation of a single element can be quantified. The fourth chapter describes the investigation of both Mg incorporation and Mg isotope fractionation in foraminifera with important implications for our understanding of the biomineralization controls by foraminifera on their calcite chemistry. The next chapter describes a field study in the Mediterranean Sea that investigates the effect of both salinity and temperature on planktonic foraminiferal oxygen isotopes, and Na and Mg incorporation. The final, sixth chapter describes how insights from the other chapters can be used to improve reconstructions of the environment in the Western Mediterranean Sea over the last 15,000 years. This chapter shows differences in the Western basin in response to the conditions leading to deposition of sapropel S1 in the Eastern Mediterranean.

Considering that growth rate has been proposed as a large influence of element/Ca levels in carbonates (Devriendt et al., 2021), in **chapter 2** we tested the effect of atmospheric CO<sub>2</sub> levels on the growth rate of *Amphistegina lessonii* and *Heterostegina depressa*. Foraminifera are among the most important calcifiers in the world's oceans, they contribute significantly to the functioning of the marine inorganic carbon cycle. With steadily rising anthropogenic atmospheric CO<sub>2</sub> levels, increasingly more carbon dioxide is dissolving the upper layers of the oceans, leading to a decrease in ocean pH. At a lower pH, calcium carbonate dissolves more easily and it becomes more energy costly to produce and maintain calcium carbonate structures that are exposed to the sea water. To quantify this effect, we cultured specimens of *A. lessonii* and *H. depressa* in four different atmospheric CO<sub>2</sub> levels and monitored their growth by regularly analysing the sea water alkalinity. We also determined growth of each individual and quantified the thickness of the carbonate formed during the experiment. Contrary to expectations, both species added more chambers at slightly elevated CO<sub>2</sub> levels and this increase in calcification also is apparent from thicker chamber walls for *A. lessonii* at moderately higher CO<sub>2</sub> levels. These results can be explained by an increased availability of inor-

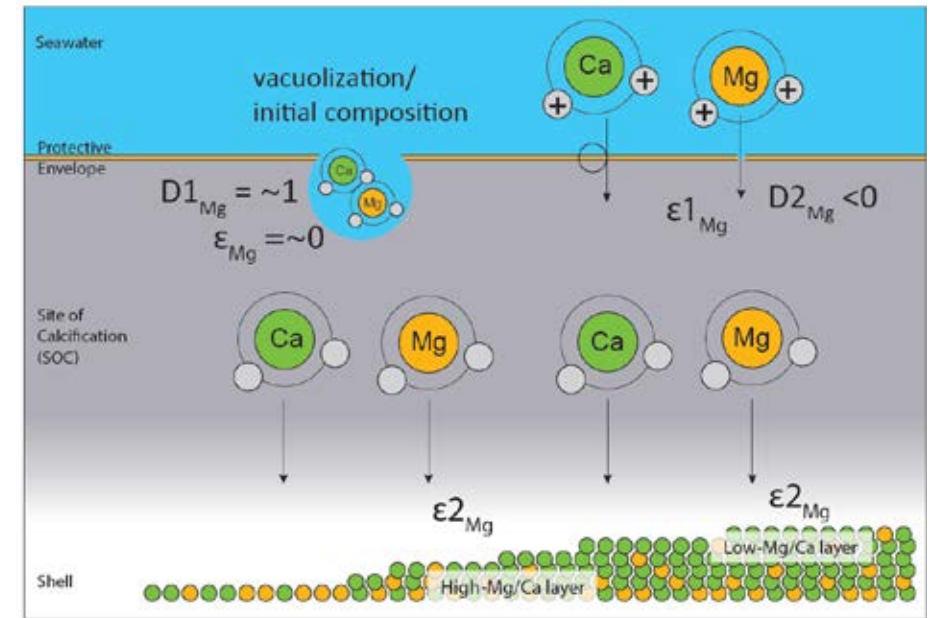


ganic carbon species to the foraminifera compared to lower atmospheric CO<sub>2</sub> levels. Only at more drastically increased CO<sub>2</sub> / lowered pH do the negative effects of ocean acidification tip the balance and overcome the positive effects, resulting in a decrease in calcification, though *H. depressa* seems to be more strongly affected by the lower pH than *A. lessonii*. This indicates that some foraminifera such as *A. lessonii* and *H. depressa* are well adapted for a moderately high CO<sub>2</sub> world and that at least some groups foraminifera might continue to thrive throughout the coming decades of rapid global changes.

We explored a potential reason for the variability observed within foraminifera populations in **chapter 3**. The tropical, large benthic, symbiont-bearing foraminifera species *A. lessonii* was used to investigate a potential cause for commonly observed inter-specimen variability. Previous studies showed that specimens grown under identical conditions still show large ranges in measured element ratios, even when they came from the same reproduction event and were thus genetically identical (de Nooijer et al., 2014b). This results in the necessity to analyse a large number of specimens from natural samples to ensure a proper representation of the sampled population. Variability of element/Ca ratios within the shell of a single specimen have previously been linked to the dark/light cycle, with bands enriched in Mg formed during night (Fehrenbacher et al., 2017; Spero et al., 2015). Here we show experimentally that not only intra-specimen variability can be caused by light conditions, but a specimen's average Mg/Ca is also highly impacted by this factor. Different individuals from the same population beginning the calcification of their next chambers at different times of the same day (though timing is not entirely random, with preferred starting times) can explain a large portion of the intraspecies variability observed in natural and cultured populations, even at constant temperatures.

To further investigate how Mg is being incorporated into foraminiferal calcite, in **chapter 4** we tested the effect of temperature on the Mg-isotope composition ( $\delta^{26}\text{Mg}$ ) of *A. lessonii*. Quantifying the Mg isotopic fractionation and combining this with overall Mg partitioning will help to understand the mechanisms responsible for Mg (and other element) incorporation and thereby improve the confidence with proxies can be applied. We exposed juvenile specimens of *A. lessonii* to three different temperatures and analysed the resulting Mg isotopic composition of their calcite shells. The results show a negative correlation between their  $\delta^{26}\text{Mg}$  and the culture temperature and when combined with previously published calibrations, suggest a two-step model (Fig. 4) for Mg-incorporation in foraminiferal calcite. This model not only incorporates commonly

observed Mg-banding in foraminifera, but it can be used to replicate the Mg isotope data published for other species simply by adjusting some of the biologically controlled parameters, which differ between species.



**Figure 4.** Foraminiferal biomineralization model including both Mg-banding previously observed as well as the relationship between  $\delta^{26}\text{Mg}$  and shell Mg/Ca. Figure taken from Dämmer et al. (2021).

In **chapter 5** we show that combining two proxies ( $\delta^{18}\text{O}$  and Mg/Ca) using the commonly used planktonic species *Globigerinoides ruber* (white) does not reflect the salinity of the seawater that the foraminifera lived in. This result was found despite using samples retrieved from an area with a large salinity gradient: Mediterranean Sea. Even though it is one of the most informative parameters for understanding global climates and ocean circulations, salinity is a notoriously difficult parameter to reconstruct. It had been proposed that the combination of Mg/Ca and  $\delta^{18}\text{O}$  as proxies could be used to achieve this. Sea water oxygen isotopes are mainly impacted by evaporation, the same factor that is driving salinity gradients in many marine settings. Though long-term temperature trends also play a major role in sea water oxygen isotopes, since colder temperatures result in removal of relatively oxygen isotopically depleted water in the form of ice and snow. Therefore, a separate proxy is needed to calculate which portion of the sea water  $\delta^{18}\text{O}$  is related to salinity and which portion is caused by temperature.

This has previously been done by measuring  $\delta^{18}\text{O}$  in foraminifera, assuming their isotopic composition reflects that of the sea water, as well as measuring their Mg/Ca ratio, which is known to correlate with sea water temperature (e.g., Breitenbach et al., 2018), with only a minor impact by other factors, such as pH or salinity. The combination of the strong natural variability in foraminiferal elemental ratios, uncertainties in all the proxy correlations and assumptions, as well as a mixing and transporting of particles due to ocean currents, unfortunately result in the combined proxy approach being not applicable to natural samples. Instead, we test the recently suggested Na/Ca as a proxy (Bertlich et al., 2018; Mezger et al., 2016; Wit et al., 2013) for salinity and find a good correlation between Na/Ca and sea water salinity, which also appears to be independent of sea water temperature. Furthermore, a strong consistency between calibrations obtained using samples from different oceans suggests a very stable relationship between these parameters for *G. ruber*.

In **chapter 6** we used a variety of proxies in benthic and planktonic foraminifera from a sediment core from the Iberian margin to investigate if evidence for past low oxygen conditions in the eastern Mediterranean Sea could be recorded in western Mediterranean Sea foraminifera through the incorporation of redox sensitive elements, such as Mn. We found that elevated Mn levels could be found in benthic foraminifera, but not in planktonic species, indicating that Mn has been transported through deep ocean currents from the east to the west. This implies a continued anti-estuarine circulation pattern in the Mediterranean Sea throughout the entire Holocene. In addition, we found stable temperatures and a stable offset between different water depth temperatures throughout the same time frame.



**Figure 5.** Standing on the Italian Island of Panarea, watching smoke (and  $\text{CO}_2$ ) erupt from Mount Stromboli. Photo credit: Caitlyn Witkowski (NIOZ)

# CHAPTER TWO

## IMPACT OF DISSOLVED CO<sub>2</sub> ON CALCIFICATION IN TWO LARGE, BENTHIC FORAMINIFERAL SPECIES

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### ABSTRACT

Rising atmospheric CO<sub>2</sub> shifts the marine inorganic carbonate system and decreases seawater pH, a process often abbreviated to ‘ocean acidification’. Since acidification decreases the saturation state for crystalline calcium carbonate (e.g., calcite and aragonite), rising dissolved CO<sub>2</sub> levels will either increase the energy demand for calcification or reduce the total amount of CaCO<sub>3</sub> precipitated. Here we report growth of two large benthic photosymbiont-bearing foraminifera, *Heterostegina depressa* and *Amphistegina lessonii*, cultured at four different ocean acidification scenarios (400, 700, 1000 and 2200 ppm atmospheric pCO<sub>2</sub>). Using the alkalinity anomaly technique, we calculated the amount of calcium carbonate precipitated during the incubation and found that both species produced the most carbonate at intermediate CO<sub>2</sub> levels. The chamber addition rates for each of the conditions were also determined and matched the changes in alkalinity. These results were complemented by micro-CT scanning of selected specimens to visualize the effect of CO<sub>2</sub> on growth. The increased chamber addition rates at elevated CO<sub>2</sub> concentrations suggest that both foraminifera species can take advantage of the increased availability of the inorganic carbon, despite a lower saturation state. This adds to the growing number of reports showing the variable response of foraminifera to elevated CO<sub>2</sub> concentrations, which is likely a consequence of differences in calcification mechanisms.

## INTRODUCTION

With globally rising atmospheric CO<sub>2</sub> levels, the marine carbonate system is steadily changing, approximately 25% of the CO<sub>2</sub> added to the atmosphere since the industrial revolution has been taken up in the upper layers of the ocean (Le Quéré et al., 2018). This uptake has decreased pH by ~0.1 units (Brewer, 1997; Caldeira and Wickett, 2003; Jiang et al., 2019; Orr et al., 2005) and shifted the speciation of dissolved inorganic carbon (DIC) by decreasing the carbonate ion concentration ([CO<sub>3</sub><sup>2-</sup>]) and increasing the bicarbonate ion concentration ([HCO<sub>3</sub><sup>-</sup>]). As a consequence, the saturation state with respect to aragonite and calcite has been steadily declining and is widely believed to hamper marine calcification, for example by increasing the energy costs for maintaining high internal saturation states (Frieder et al., 2017; McCulloch et al., 2012). However, the addition of CO<sub>2</sub> to seawater also elevates the total concentration of DIC, which may be beneficial to calcification provided that the organism is capable of manipulating the ratio between the different inorganic carbon species. This may be achieved, for example, by actively increasing the pH during calcification and hence converting the (extra) HCO<sub>3</sub><sup>-</sup> into CO<sub>3</sub><sup>2-</sup>, thereby increasing saturation state (Comeau et al., 2017; de Nooijer et al., 2009). Differences in the ability to manipulate their internal pH, may explain the observed variable responses of organisms to ocean acidification (Liu et al., 2020; Ries et al., 2009). Other parameters that may determine the reaction of foraminifera to ocean acidification may include increased carbon uptake by the symbionts (if present) and increased energy allocation to maintain the intracellular-extracellular ion balance (Ries, 2011).

Foraminifera are amongst the ocean's most important calcifiers, with planktonic species estimated to produce up to 50% of all calcium carbonate in the open ocean (Schiebel, 2002). In tropical regions, large benthic foraminifera can contribute up to 54% of the sediment (Dawson et al., 2014; Hallock, 1981; Langer et al., 1997) and approximately 80% of foraminiferal derived carbonate in reefs stems from large benthic foraminifera (Langer et al., 1997). Since both planktonic and benthic foraminifera play a significant role in the global calcium carbonate production, it is essential to quantify and understand their response to changes in marine inorganic carbon chemistry. Since calcification produces CO<sub>2</sub>, the net impact of ocean acidification on calcification rates may either provide a positive or negative feedback to atmospheric CO<sub>2</sub>.

Among the larger benthic foraminifera the responses to ocean acidification are mixed (Narayan et al., 2022), with reports showing a reduction in calcification (Kuroyanagi et al., 2009, 2021; Prazeres et al., 2015; Reymond et al., 2013b, 2013a; Schmidt et

al., 2014; Sinutok et al., 2011) or standing stocks (Martinez et al., 2018; Uthicke et al., 2013), but also an increase in chamber addition rates or no response to experimentally-induced ocean acidification (Prazeres et al., 2015; Reymond et al., 2022; Stuhr et al., 2021). Whereas the response in growth rates by low Mg-foraminifera to changes in *p*CO<sub>2</sub> seems to be less variable (Dissard et al., 2010), the overall mixed responses of foraminiferal calcification may well indicate differences in biomineralization strategies between genetically distant groups (de Nooijer et al., 2014a). This could be related to the presence of photosynthetic symbionts in most large benthic foraminifera and their absence in most smaller, benthic foraminifera. Photosynthesis and calcification are linked in multiple ways: both processes compete for inorganic carbon, but the local elevation of pH by the symbiont's photosynthesis may also facilitate calcite precipitation through a higher saturation state. If the stimulus of photosynthesis is more important than the competition for inorganic carbon, the presence of symbionts may affect Mg incorporation by elevation of the precipitation rate (Burton and Walter, 1991; Mavromatis et al., 2013). Manipulation of the internal pH is so far only recorded for species precipitating low Mg/Ca calcite, which may indicate that the strong fractionation against Mg in many rotaliid foraminifera is a direct consequence of a calcifying fluid that is well-separated from ambient seawater (Nehrke et al., 2013). Species with a Mg/Ca closer to those found in inorganic precipitation experiments, may well precipitate from a more seawater-like fluid; the absence of a strong pH gradient (high inside, low outside; (Toyofuku et al., 2017)) may reflect a regular exchange of the calcifying fluid with the surrounding seawater (Erez, 2003).

Here, we test the effect of elevated *p*CO<sub>2</sub> on calcification in the large benthic foraminifera *Amphistegina lessonii* and *Heterostegina depressa*. These species both occur in shallow, tropical regions but differ in the chemical composition of their calcite. The former species precipitates a shell consisting of layers (lamella) with an intermediate Mg/Ca ratio (~40 mmol/mol; (Mewes et al., 2015)). *H. depressa* also belongs to the Rotaliida, but belongs to a different superfamily of which the members precipitate calcite with a higher Mg/Ca (~150 mmol/mol; (Raitzsch et al., 2010)). We incubate both species across a range of atmospheric *p*CO<sub>2</sub> concentrations (400-2200 ppm) and determine net calcification by monitoring changes in alkalinity. Moreover, we cross-calibrate the alkalinity changes with variability in chamber addition and, for *A. lessonii*, chamber wall thickness measured using MicroCT-scanning. The combination of these results can be used to increase our understanding of the interactions between marine inorganic carbonate chemistry and foraminiferal calcification, and could therefore help predicting and understanding the reaction of one of the most important calcifiers, large benthic foraminifera, to future climate change.



## MATERIAL AND METHODS

### SAMPLE COLLECTION AND CULTURE

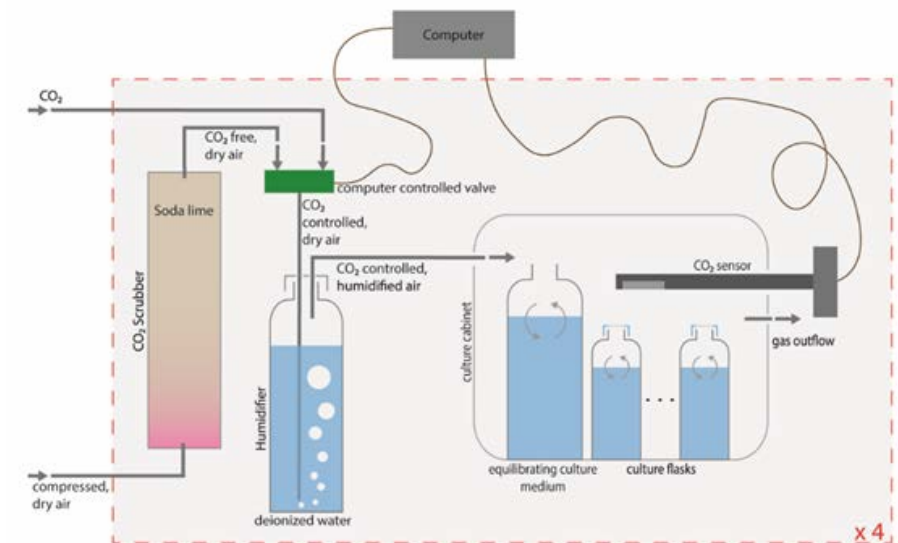
Specimens of the large benthic foraminifera *Amphistegina lessonii* and *Heterostegina depressa* were isolated from sediment collected at the tropical reef aquarium at Burgers' Zoo in Arnhem, The Netherlands (Ernst et al., 2011). Living specimens were identified by their homogeneously coloured yellow/brown cytoplasm, pseudopodial activity and motility of specimens. After isolation, they were divided into groups of 50 (*A. lessonii*) or 25 (*H. depressa*) specimens of comparable sizes (approximately 500-600µm diameter for *A. lessonii*) and placed in flasks filled with 50 ml of filtered (5µm) North Atlantic sea water. For each of the two species, one flask per CO<sub>2</sub> condition contained 5 mg/L fluorescent calcein indicator to stain newly formed carbonate. A solution of freeze-dried *Dunaliella salina* was added to all flasks, that were placed at 24°C for seven days. After this pre-staining period, calcein-marked specimens were examined under a Zeiss Axioplan 2 microscope, equipped with appropriate excitation and emission optics to identify newly formed chambers. Those individuals with one or more fluorescent chambers were placed back into the culturing flasks and the calcein-containing media were replaced with filtered, calcein-free seawater. This water was exposed to an atmosphere corresponding to that of the experimental condition to allow for pre-equilibration of the carbonate system in the water for one week.

This procedure resulted in an experiment with four groups of three replicates for *A. lessonii* and four groups of two replicates (or duplicates) for *H. depressa*. The 50 or 25 specimens within one flask are considered pseudo-replicates and variability within as well as between culture flasks will be separately discussed to test for the potential effect of pseudo-replication. For *H. depressa*, one of the flasks during the pre-staining phase did not contain any specimens with stained chambers and therefore counting the number of chambers added was not possible, but calcification can still be compared with the other treatments through alkalinity measurements.

### CULTURING SETUP

The culture set-up (Fig. 1) consisted of four chambers with controlled atmospheric composition of 18.5 L air volume. Each chamber contained a Vaisala CARBO-CAP GMD20 sensor placed in the centre of the chamber, constantly monitoring atmospheric CO<sub>2</sub> concentrations. Custom-made software (available from the authors upon

request) based on Siemens Simatic Step 7 software automatically calculated the ratio between CO<sub>2</sub>-scrubbed, compressed, dried air and pure CO<sub>2</sub> that needed to be added to maintain the pre-set atmospheric CO<sub>2</sub> concentrations, while keeping a constant (3 Lmin<sup>-1</sup>) gas flow rate. The four treatments were set to CO<sub>2</sub> levels of 400, 700, 1000 and 2200 ppm atm pCO<sub>2</sub>. Measured CO<sub>2</sub> levels were typically within +/-10ppm for the higher concentrations and within +/-40ppm for the lower concentrations. After opening the cabinets, which was necessary to for example take water samples, CO<sub>2</sub> levels returned to the set values within minutes.



**Figure 1. CO<sub>2</sub>-controlled culture set up.** A control unit constantly monitored the pCO<sub>2</sub> in four cabinets and compares them to set values. According to the offset, the ratio between pure CO<sub>2</sub> and CO<sub>2</sub>-free air was adjusted and once humidified, added to the cabinet. This secured an atmosphere with constant pCO<sub>2</sub> for the period of the culturing experiment. This schematic shows just one set up, everything shown within the red rectangle (CO<sub>2</sub> scrubber, humidifier, and culture cabinet) was build four times and used in parallel for the experiment connected to just one computer, with a different pCO<sub>2</sub> in each of the four chambers. The different components are not to scale.

The gas mixture was bubbled through a humidifying chamber filled with 1 L of de-ionized water before entering the cabinet to elevate the humidity of the gas inside the cabinet, since a very low humidity would result in high evaporation, which in turn would change salinity and other parameters. To minimize evaporation from the culturing flasks even further, flasks were closed with vented lids that allow for gas exchange.

Several additional open bottles filled with 250 ml of filtered sea water were placed into each chamber to allow equilibration between the dissolved CO<sub>2</sub> in these bottles' seawater and that of the atmosphere.

These equilibrated bottles were used weekly to replace the culture media in the culturing flasks and ensured constant carbonate chemistry during the incubation period. After subsampling (see section below, 'Carbonate chemistry of the culture media'), any remaining culture medium was discarded and replaced by fresh, pre-equilibrated culture medium. All foraminifera were then fed by adding 1 ml of a concentrated solution containing freeze-dried *D. salina*. This concentrated solution was prepared by suspending 32.5 mg of the algae in 100 ml of the corresponding treatment's pre-equilibrated culture medium.

Salinity in the culture media was monitored and constant at 37.2. Temperature was kept at 23 °C, light was switched on for 12 hours per day to simulate a natural day/night light cycle using fluorescent tubes with a light intensity of 240 μmolm<sup>-2</sup>s<sup>-1</sup>, close to that experienced in the field by both *Amphistegina* sp. and *H. depressa* (Nobes et al., 2008), and a luminous flux of 300 lux. The transparent culture cabinets were illuminated from four sides to ensure homogeneous light conditions.

After 36 (for *H. depressa*) or 50 days (for *A. lessonii*), the experiment was terminated by rinsing the culture flasks with de-ionized water and removing the specimens with a brush. Foraminifera tests (i.e., shells) were treated following a cleaning protocol (Barker et al., 2003; van Dijk et al., 2017b). Examination with fluorescent microscopy was repeated to count newly formed chambers, identified by non-stained chambers formed after those stained by calcein.

Since not all specimens used in this experiment were calcein-stained, only chamber addition of pseudo-replicates in one flask were used to calculate the total population's growth for each treatment. The percentage of calcein-stained specimens that did not calcify during the experiment in each treatment was between 20 and 30%. Since only one of the triplicates/duplicates for each treatment was stained, to estimate the total number of calcifying specimens (including in those flasks that were not pre-stained) in each treatment, the mortality was extrapolated from the pre-stained flasks to all flasks. Between 20 and 30% of all specimens were assumed to not have formed any chambers, therefore this percentage (i.e. 25%) was subtracted from the total number

of specimens. The average number of chambers formed by stained, calcifying specimens was extrapolated to the entire population of the same species and treatment (Table 2). The total number of chambers formed was calculated using the average number of chambers formed by calcifying calcein-stained specimens as representative of the entire population of the treatment.

## CARBONATE CHEMISTRY OF THE CULTURE MEDIA

Every week, samples were collected to measure dissolved inorganic carbon (DIC), nutrients (PO<sub>4</sub>, NH<sub>4</sub>, NO<sub>3</sub>, NO<sub>2</sub> and SiO<sub>2</sub>) and for Total Alkalinity (TA) were collected from the replaced culture media. From these parameters, the entire inorganic carbon system can be calculated. Due to the small water volumes used per replicate, water samples from culturing flasks from the same incubator were combined into one aliquot to ensure sufficiently large volume for the analyses. Samples for DIC were stored in airtight 5 mL glass vials poisoned with 15 μL mercury (II) chloride (HgCl<sub>2</sub>) prior to analysis.

Nutrients were analysed in a temperature-controlled laboratory equipped with a TRAACS Gas Segmented Continuous Flow Analyser. Measurements were made simultaneously on four channels for Phosphate, Ammonium, Nitrite, and Nitrate + Nitride and measured following established protocols (Grasshoff et al., 1978; Helder and de Vries, 1979; Murphy and Riley, 1962; Strickland and Parsons, 1972). All measurements were calibrated with standards diluted in low nutrient seawater with a salinity of 37 to ensure that analyses were performed within the same matrix as the samples.

DIC was measured using a Technicon TRAACS 800 autoanalyzer spectrophotometric system (Stoll et al., 2001). TA was measured using an automated spectrophotometric alkalinity system described by Liu et al. (2015). To summarize, 45 μL of bromocresol purple (10 mmol/L), which changes its colour with pH, was added to 60 ml of the sample, the mixture was then titrated with 0.1 M HCl, and the resulting changes in colour were monitored by spectrophotometry. CRM (Certified Reference Material, Dr. Dickson, Scripps Institution of Oceanography) was used as a standard material for drift correction, the measured nutrients from the same sample were used to correct the TA. Standard deviation between replicate measurements was typically ~3 μmol/kg.

The Alkalinity Anomaly Technique allows calculating calcification (Smith and Key, 1975). The precipitation of 1 mol of calcium carbonate causes a decrease of 2 moles

TA (Zeebe and Wolf-Gladrow, 2001). The changes in TA observed between the beginning of the incubation period and at the end of the experiment is thus proportional to the amount of  $\text{CaCO}_3$  precipitated during that period. This result needs to be corrected for other factors, which can also alter TA in sea water, such as changes nutrient concentrations and salinity. Therefore, the following equation (Jacques and Pilson, 1980) was used to assess the amount of  $\text{CaCO}_3$  formed each week by each species in each treatment:

$$m(\text{CaCO}_3) = 0.5 \times [\Delta\text{TA} + \Delta\text{PO}_4 - \Delta\text{NH}_4 + \Delta(\text{NO}_3 + \text{NO}_4)] \times V_{\text{SW}} \times \rho_{\text{SW}} \times M(\text{CaCO}_3)$$

where  $V_{\text{SW}}$  is the volume of sea water used for the TA measurements in L,  $\rho_{\text{SW}}$  is the density of the sea water corrected for salinity and  $M(\text{CaCO}_3)$  is the molar mass of  $\text{CaCO}_3$ . The result is  $\text{CaCO}_3$  (in  $\mu\text{g}$ ) precipitated by the foraminifera. The remaining carbonate system parameters (pH and saturation state  $\Omega$ ) were calculated with PyCO2SYS v1.6.0 (Humphreys et al., 2022). The decision which of the carbonate system parameters were to be measured and which were to be calculated was based on the experimental design and technical restrictions.

## MICROCT-SCANNING OF CULTURED FORAMINIFERA

A total of 19 calcein-stained *A. lessonii* specimens were analysed using high resolution micron-scale computed tomography (MicroCT; Zeiss Xradia 520 Versa) at Naturalis Biodiversity Center (Leiden, the Netherlands). Specimens were imaged at 80 kV using 4\* optical magnification at a voxel size of 1.1-1.5  $\mu\text{m}$ . and introduced into Avizo 2020.3 3D software (ThermoFisher Scientific, Waltham, MA, United States) to generate 3D models of the tests and segmentation of the chambers grown during the experiment.

Using the MicroCT-scans, a 3D-rendering of each scanned specimen was created with Fiji (ImageJ 1.53c). For each specimen, an orientation in the 3D-rendering was found, that sectioned the shell wall of the F-chamber at a 90° angle, using the pores as a guide. The correct plane was identified by pores that penetrate the entire section of the shell wall at a constant width. The correct plane was then used to measure shell wall thickness by seven randomly placed lines drawn between the outer and inner chamber wall's surfaces. In addition, for two specimens (one grown at 700 and one grown at 2200 ppm  $p\text{CO}_2$ ) the volume of carbonate in the F-chamber was estimated based on the MicroCT-scans. To compare with previously published results on calcification (Segev and Erez, 2006), the volumes ( $\text{mm}^3$ ) were converted to weight ( $\mu\text{g}$ ) using the density of calcite ( $2.71 \text{ g/cm}^3$ ).

## STATISTICAL TREATMENT OF THE DATA

To test for significant differences in the average chamber addition rates and average changes in TA as a function of  $p\text{CO}_2$ , a t-test was performed on combinations of two groups for each of the species (four groups in total for *A. lessonii* and three in case of *H. depressa*). The type of t-test applied assumed independent samples, since the compared data (i.e., specimens from different bottles) are not related.

An ordinary least sum of square regression analysis was performed to investigate the relationship between the number of chambers formed during the experiment and the calculated amount of carbonate produced based on the change in alkalinity. A linear regression model was assumed and significance (p-value) as well as the equation describing the regression and the residual sum of squares ( $R^2$ ) are reported. The calculated 95% confidence interval for the linear regression is also plotted.

## RESULTS

### INORGANIC CARBONATE SYSTEM

The inorganic carbonate system of the culture media at the beginning of the culturing period (Table 1) shows that even at the highest  $\text{CO}_2$  concentrations (2200 ppm), the culture media remained saturated with respect to calcite ( $\Omega > 1$ ).

**Table 1. Seawater inorganic carbon chemistry at the onset of the experiment.** Total alkalinity (TA) and dissolved inorganic carbon (DIC) concentration were measured and used to calculate pH and saturation state  $\Omega_{\text{Calcite}}$ .

measured					calculated	
Salinity	Temperature (°C)	$p\text{CO}_2$ (ppmv)	TA ( $\mu\text{mol/kg}$ )	DIC ( $\mu\text{mol/kg}$ )	pH (total scale)	$\Omega_{\text{Calcite}}$
37	23	400	2395	2080	8.05	5.22
37	23	700	2411	2210	7.85	3.55
37	23	1000	2411	2270	7.71	2.73
37	23	2200	2403	2392	7.36	1.30



Weekly changes in the total alkalinity varied greatly between species and CO<sub>2</sub> concentrations (Figure S1, Table S1). For both species the changes in TA follow a similar pattern with the strongest decrease in TA occurring at 700 ppm pCO<sub>2</sub> and the smallest decrease at the highest pCO<sub>2</sub>.

## FORAMINIFERAL CHAMBER ADDITION RATES

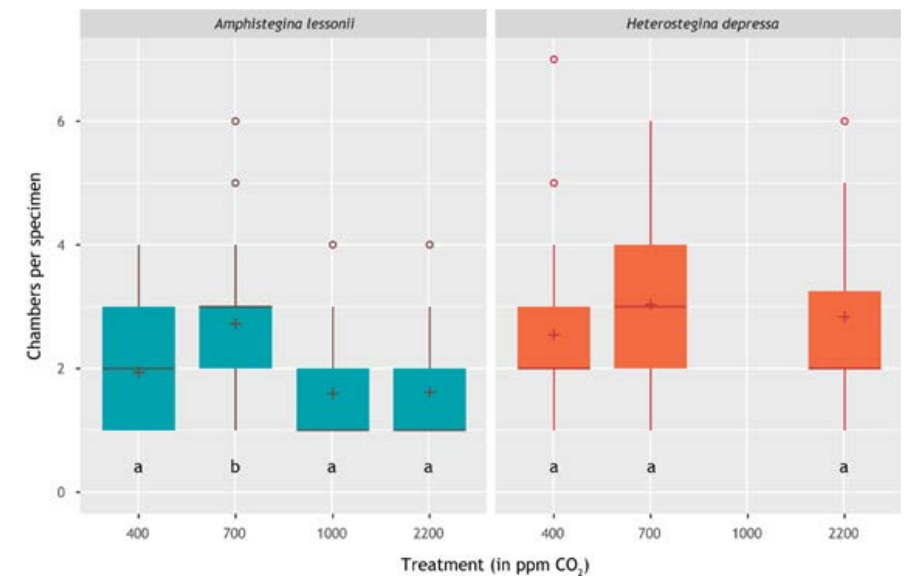
More than 70% of the *Amphistegina* specimens and almost 70% of all *Heterostegina* specimens continued adding chambers to their shell during the culturing experiment, with the highest percentage (85 %) of calcifying *A. lessonii* specimens at 700 ppm. For *H. depressa*, there is no clear relation between the portion of calcifying specimens and the pCO<sub>2</sub> (Table 2, Figure S2).

Similar mortality rates (20-30%) have been observed in previous culturing experiments (van Dijk et al., 2017a) and are likely due to the transfer of specimens to other conditions (e.g., from the stock aquarium into a petri dish and then into the culture flasks). Since there is no relation between mortality and pCO<sub>2</sub> of the experimental condition, survival is unlikely due to the change in pCO<sub>2</sub> itself.

**Table 2 Number of chambers added per specimen during the experiment, estimated number of calcifying specimens and extrapolated number of chambers added during the experiment for both species cultured.** Results for *H. depressa* incubated at 1000 ppm of CO<sub>2</sub> are not available since too few of them grew chambers during pre-staining. Note the different experiment durations for both species, 50 days for *A. lessonii* and 36 days for *H. depressa*.

pCO <sub>2</sub> ppm	Chambers added/ specimen (+/- 1SD)				Calcifying specimens		Chambers added	
	<i>A. lessonii</i>		<i>H. depressa</i>		<i>A. lessonii</i> (n = 150)	<i>H. depressa</i> (n = 50)	<i>A. lessonii</i>	<i>H. depressa</i>
400	1.94 (± 0.88)	n=31	2.55 (± 0.88)	n=11	108 (72%)	32 (64%)	210	82
700	2.73 (± 1.23)	n=30	3.03 (± 1.45)	n=15	128 (85%)	33 (66%)	349	100
1000	1.60 (± 0.73)	n=30	NA	NA	104 (69%)	NA	166	NA
2200	1.62 (± 0.85)	n=23	1.62 (± 0.85)	n=12	83 (55%)	38 (76%)	134	62

For the calcifying specimens, the number of chambers that were added varied between 1 and 6 for *A. lessonii* and between 1 and 7 for *H. depressa*. Variability in chamber addition rates for the pseudo-replicates (i.e., variability between number of chambers added per specimen from one flask) was always between 45 and 55% (relative standard deviation). Combined, this shows that the total number of chambers added is more than 2.5 times higher in the 700 ppm pCO<sub>2</sub> treatment compared to 2200 ppm pCO<sub>2</sub> for *A. lessonii* and 1.6 times higher for *H. depressa* (Table 2; Fig 2). For both species, the extrapolated number of added chambers in the 400 ppm treatment is between the number of chambers added at 700 ppm and 2200 ppm. Chamber addition rates could not be quantified for *H. depressa* in the 1000 ppm treatment due to lack of calcein stained specimens. *A. lessonii* appears to add most chambers at the second lowest pCO<sub>2</sub> (700 ppm) and least in the highest pCO<sub>2</sub> (2200 ppm) treatment.

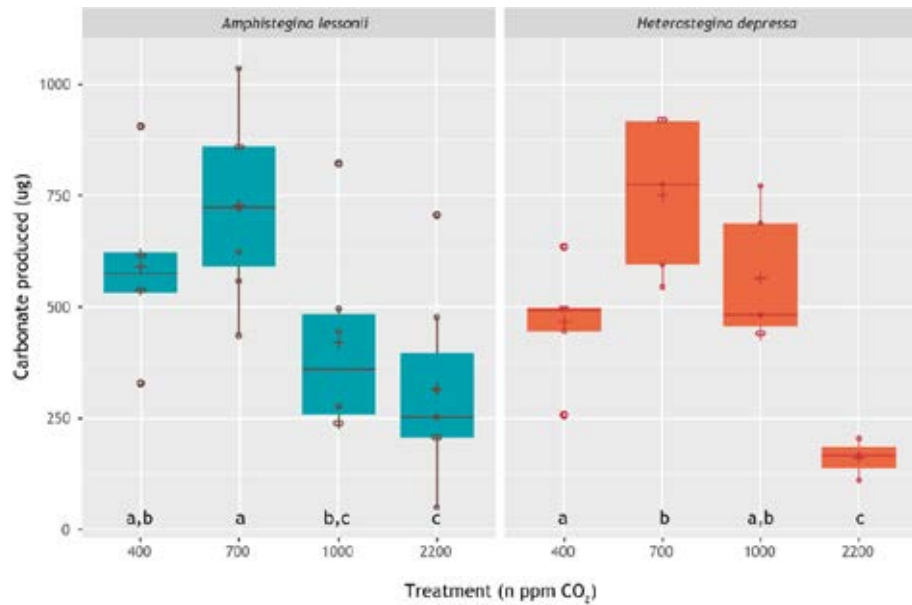


**Figure 2. Box plot showing the number of chambers added per calcein-stained, calcifying individual (specimens with no chambers were excluded).** Average values are indicated by plus symbols. Letters below the boxplots indicate statistically significantly different groups (t-test, p-value < 0.05).

## CHANGES IN CALCIFICATION

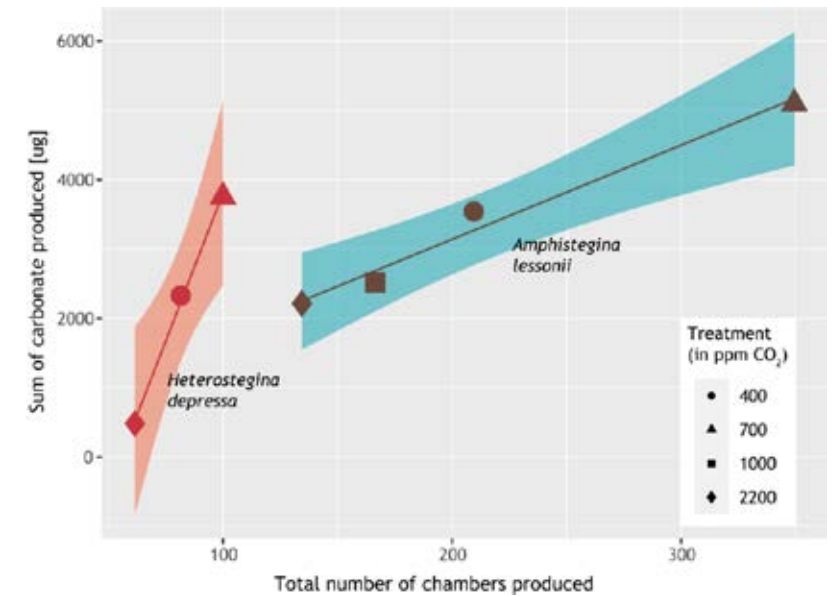
Both species produced most new carbonate (~750 µg) at pCO<sub>2</sub> levels of 700 ppm. Moreover, the total amount of new carbonate added was very similar between the two species (Fig 3). Incubated specimens of *H. depressa* produced notably less new carbon-

ate (approximately 150  $\mu\text{g}$ ) at the highest  $p\text{CO}_2$  levels (2200 ppm), whereas for specimens of *A. lessonii*, calcification was similar at 1000 and 2200 ppm (250-350  $\mu\text{g}$ ).



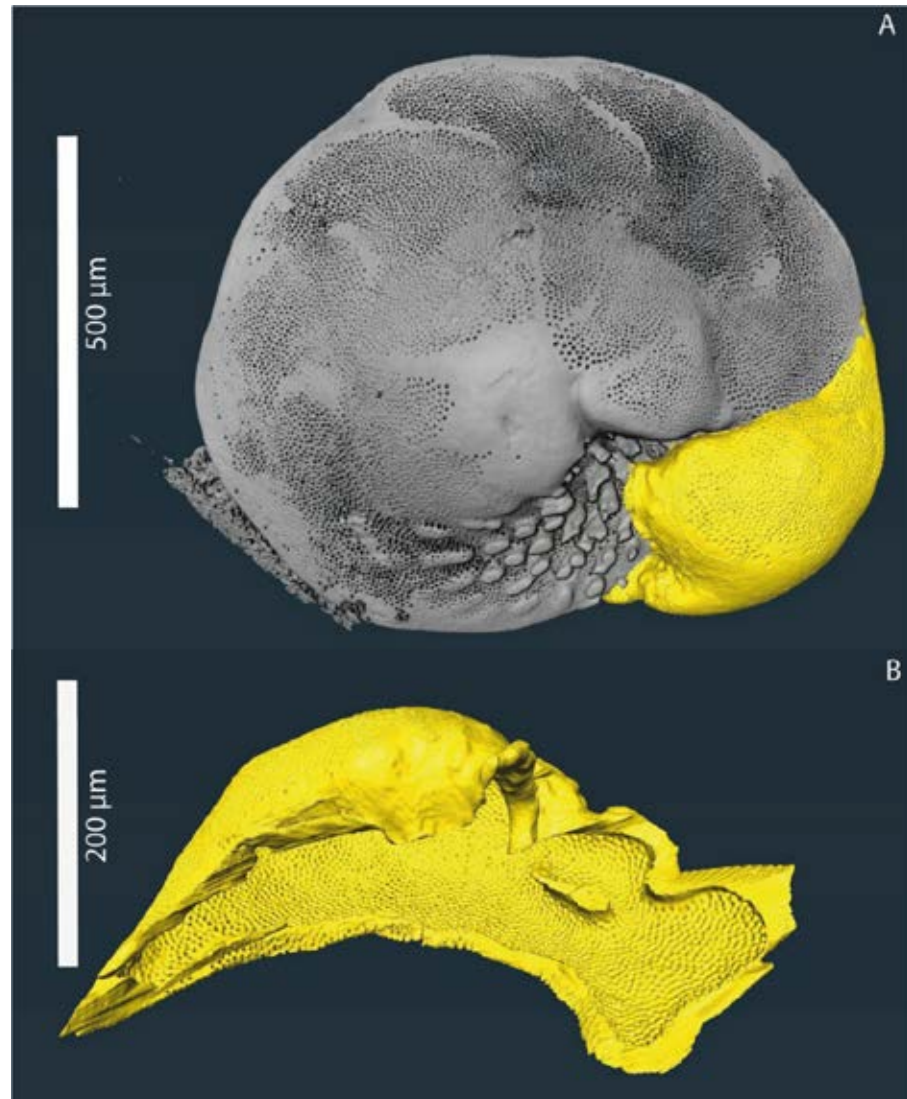
**Figure 3. Total amount of carbonate formed in each treatment per week, calculated from weekly changes in TA.** Statistically identical ( $t$ -test,  $p$ -value > 0.05) treatments are indicated by the same letter. *A. lessonii* calcifies significantly less under higher  $\text{CO}_2$  concentrations (1000 and 2200 ppm) than at ambient and moderately increased levels (400 and 700 ppm). *H. depressa* calcifies significantly less at highest  $\text{CO}_2$  concentrations (2200 ppm) compared to all other treatments. Both species appear to have a calcification optimum at slightly elevated  $p\text{CO}_2$  levels.

The sum of carbonate produced over the entire experiment can be compared with the number of chambers formed during the experiment (Fig. 4), showing positive correlations, albeit that the slope of this dependency is steeper for *H. depressa* than for *A. lessonii*.

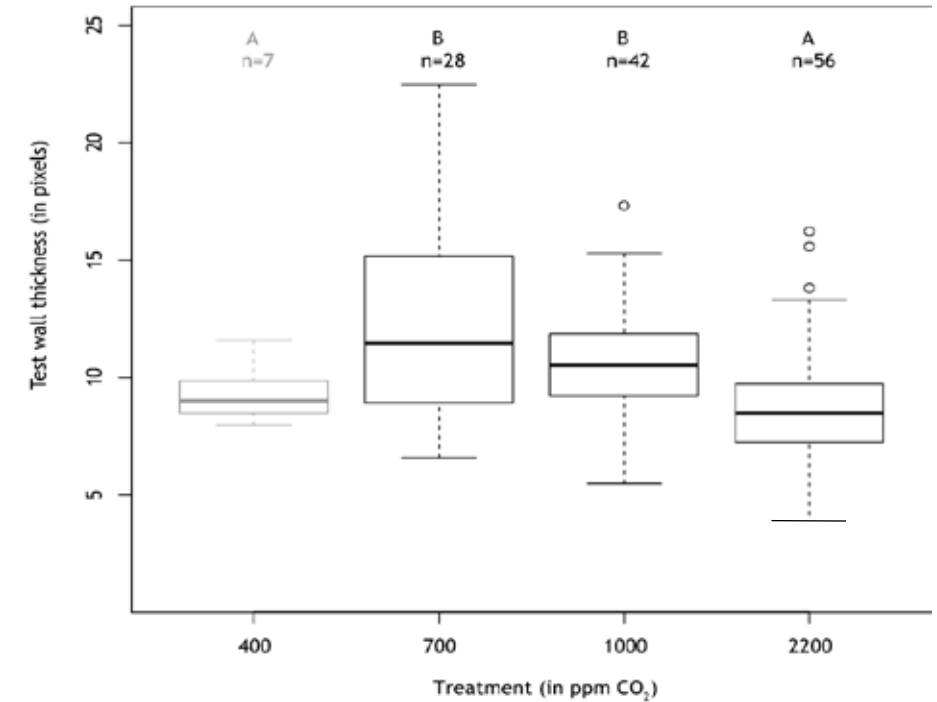


**Figure 4. Relationship between the amount of carbonate and the number of chambers produced.** The amount of carbonate formed by the foraminifera correlates linearly with the total number of chambers formed ( $p$ -values < 0.05). The relationship is described by  $y = 85.27(\pm 4.26)x - 4722.67(\pm 351.65)$  for *H. depressa* (adjusted  $R^2 = 0.995$ ) and by  $y = 13.57(\pm 1.43)x + 424.48(\pm 325.22)$  for *A. lessonii* (adjusted  $R^2 = 0.968$ ). Both species produce the highest amount of carbonate and the largest number of chambers at 700 ppm  $p\text{CO}_2$  and the lowest amount of carbonate and the lowest number of chambers at 2200 ppm  $p\text{CO}_2$ .

Similar to the number of chambers, the average test wall thickness, determined from MicroCT-Scans (Fig. 5), in *A. lessonii* also varies with  $p\text{CO}_2$ , with thickest walls at  $p\text{CO}_2$  levels of 700 and 1000 ppm (Fig. 6). Based on isolation of the complete final chamber from the MicroCT-scan, the volume of calcite of the F-chamber from a single specimen from the 700 ppm treatment was determined to be close to 300,000  $\mu\text{m}^3$ , which equals approximately 8 nmol of  $\text{CaCO}_3$ . In one specimen from the 2200 ppm treatment, the added calcite amounted to 47 nmol. The associated change in TA for this addition is lower than the total observed decrease in TA (Table S1) by 2 and 10-fold at 2100 and 700 ppm, respectively.



**Fig 5. 3D-Rendering of an *Amphistegina lessonii* specimen.** An X-ray photograph of an *A. lessonii* specimen, the F-chamber is highlighted in yellow (Panel A). The F-chamber was virtually separated from the foraminifer to calculate the volume of the carbonate (Panel B).



**Fig 6. Dependence of test wall thickness on pCO<sub>2</sub>.** The test wall thickness of F-chambers of *A. lessonii* specimens grown during the experiment varies with atmospheric pCO<sub>2</sub> levels. Specimens exposed to moderately elevated CO<sub>2</sub> concentrations show on average thicker shell walls than specimens grown under low as well as very high CO<sub>2</sub> concentrations. Letters above the boxplots indicate statistically significantly different groups (t-test, p-value < 0.05). The 400 ppm treatment is shown in grey, due to the comparatively small number of thickness measurements used.

## DISCUSSION

### CALCIFICATION IN FORAMINIFERA AS A FUNCTION OF pCO<sub>2</sub>

A moderate increase in dissolved CO<sub>2</sub> had a positive effect on calcification, both for *Amphistegina lessonii* and *Heterostegina depressa*. Most chambers were added at 700 ppm (Table 2) and for *A. lessonii*, most specimens (85%; Table 2) calcified at this pCO<sub>2</sub>. The total amount of carbonate produced, based on the changes in TA, was also highest for both species at 700 ppm pCO<sub>2</sub> (Fig. 3) and for *A. lessonii*, newly added chamber walls were thicker at 700 and 1000 ppm (Fig. 6).

At the highest CO<sub>2</sub> concentration (2200 ppm), both species added the least number of new chambers (Table 2) and the chamber walls of *A. lessonii* were thinnest at 400

and 2200 ppm (Fig. 6). Our results show that determining foraminiferal calcification by counting new chambers, determining the chamber wall's thicknesses and by monitoring the decrease in alkalinity, all reveal a similar response of calcification to  $p\text{CO}_2$ . It may be that calcification does not scale with  $p\text{CO}_2$  linearly (or parabolically), but that the direction in the change of the saturation state also matters. Such hysteresis (McMahon et al., 2013) is reported for coral reefs and is visible on daily changes in pH due to photosynthesis. Such a potential effect in foraminifera will be harder to find due to the intermittent nature of calcification in rotaliid foraminifera, but we cannot exclude the possibility that hysteresis may influence their chamber addition process.

The moderately positive influence of  $\text{CO}_2$  on calcification as observed here is in contrast with the response of the majority of marine calcifiers (Kroeker et al., 2013) and reports on the effect of  $\text{CO}_2$  on larger benthic foraminifera (Doo et al., 2014). Although other studies have found mixed responses of large benthic foraminifera to elevated  $p\text{CO}_2$ /reduced pH (McIntyre-Wressnig et al., 2013; Narayan et al., 2022) and showed that they are able to withstand periodic undersaturated conditions (Charrieau et al., 2022). Finally, Fujita et al. (2011) reported an optimum growth rate for three species of the large, high-Mg/Ca benthic foraminifera, similar to the pattern reported here. Although the number of incubated and analyzed specimens is smaller for *H. depressa* than *A. lessonii*, sensitivity to  $p\text{CO}_2$  of the latter appears strongest (Table 2). This is in line with a previous study reporting no changes in growth rates in *H. depressa* at  $p\text{CO}_2$  values between 467 and 1952 ppm (Vogel and Uthicke, 2012), possibly indicating a strong decrease in calcification in this species for high (>2000 ppm)  $\text{CO}_2$  concentrations.

The (positive) response of large benthic foraminifera to elevated  $p\text{CO}_2$  likely involves the photosynthetic symbionts residing inside the shells of these foraminifera (diatoms for genera like *Amphistegina*, dinoflagellates for genera like *Amphisorus*; Hikami et al., 2011; Maeda et al., 2018). The additional  $\text{CO}_2$  likely increases the delivery of nutrients and carbon from the symbionts to the foraminifera (Ter Kuile and Erez, 1987) and thereby provides an indirect link between carbon dioxide and calcification. Any negative impact (e.g., by increasing the energy costs associated with the raised intracellular-extracellular pH gradient) by the  $\text{CO}_2$ -induced acidification may not outweigh the positive influence of enhanced photosynthetic activity. Furthermore, if these effects do not scale linearly with the concentration of  $\text{CO}_2$ , pH or saturation state, their added effect may give rise to a net hyperbolic response of calcification to  $p\text{CO}_2$ .

For smaller benthic and planktonic foraminiferal species, many studies indicated a negative response of calcification (either growth rate or size-normalized weight) even with a modest increase in  $p\text{CO}_2$  (Allison et al., 2010; Barker and Elderfield, 2002;

Bijma et al., 1999, 2002; Dissard et al., 2010; Gonzalez-Mora et al., 2008; Keul et al., 2013; De Moel et al., 2009). These results may indicate that either calcification itself is affected by higher  $p\text{CO}_2$ /lower saturation states or that other metabolic processes are hampered by elevated  $p\text{CO}_2$  and that calcification is indirectly affected. These studies, however, all focused on low-Mg/Ca species (e.g., planktonic foraminifera or *Ammonia* spp.).

The overall clear effect of elevated  $p\text{CO}_2$  on low-Mg benthic and planktonic foraminifera compared to the mixed, moderate, or even positive response by large benthic foraminifera may indicate that the mechanism responsible for incorporating Mg (or the lack thereof) is related to the pathway through which the inorganic carbon is taken up for calcification. This is in line with reports of inorganic carbon manipulation experiments on the incorporation of cations (Dissard et al., 2010; Dueñas-Bohórquez et al., 2011) and was more recently provided with a mechanistic basis in which the inward calcium pumping is directly responsible for the uptake of inorganic carbon (Toyofuku et al., 2017). The coupling between inorganic carbon uptake and Mg partitioning would explain the much higher sensitivity of the low Mg/Ca planktonic foraminifera to seawater carbonate chemistry at relatively low  $p\text{CO}_2$  values (Bijma et al., 1999). Alternatively, the distinction between these foraminifera in their response to elevated  $p\text{CO}_2$  may be a function of their ecology and/ or presence of photosynthetic symbionts.

## CHAMBER ADDITION VERSUS CALCIFICATION

At intermediate  $p\text{CO}_2$  values (700 ppm), the two foraminifera species investigated here produce most  $\text{CaCO}_3$ . Although chamber addition rate (Fig. 2) has been hypothesized to primarily reflect growth of the protoplasm which resides in the shell (Berger et al., 1978), we here observe a good correlation with shell wall thickness and alkalinity change. Protoplasmic growth likely follows food uptake, but here we also find a relation with carbonate chemistry of the water. This suggests that the cost of calcification is affecting the overall well-being of the foraminifera, potentially through the energetic cost of calcification especially at low pH (Ries, 2011; Zeebe and Sanyal, 2002). When a foraminifer is calcifying relatively well (i.e., produces a thick chamber wall) the reduced energetic costs may result in increased growth and hence also chamber addition. This may imply that chamber addition itself is hence indirectly coupled to calcification rate.

When comparing the precipitated calcite as calculated from the reduction in alkalinity and the number of chambers added, each chamber would consist of 10 to 15 micrograms of calcite (Fig. 4). This is much more than the approximately 1 microgram of carbon estimated by the weight of individual tests divided by the average number of

chambers making up the shell (average weight of adult *Amphistegina* (Segev and Erez, 2006)). As the last chambers in rotaliid species are larger and potentially contain relatively much calcite, we also calculated the volume of the carbonate added for two selected MicroCT-scanned specimens. This confirmed the offset between alkalinity based total amount of carbonate precipitated and the estimated carbonate based on chamber addition. The offset appears too large to be explained with factors such as the carbonate added on the older parts of the shell in addition to the added chamber itself (i.e., by lamellar calcification; (Reiss, 1957)). The fact that some of the specimens were more robust than others does not explain the observed offset either. Still, the evident correlation between the alkalinity and shell thickness also suggests a common response to the imposed carbonate chemistry. This suggests that either inorganically within the culture flask, or biomediated through micro-organismal calcification, the overall calcification in the culture flasks supplemented the foraminiferal calcification rate changes.

## CALCIFICATION IN A FUTURE OCEAN

A calcification optimum of large benthic foraminifera at increased  $p\text{CO}_2$  levels would result in increased calcification with rising anthropogenic atmospheric  $\text{CO}_2$  concentrations within the next decades, which in turn would lead to a weak, but significant, positive feedback loop with  $\text{CO}_2$  concentrations (Humphreys et al., 2018), since calcification is a net source for dissolved  $\text{CO}_2$  (Frankignoulle et al., 1994). Due to the interplay of acidification, warming and pollution it is difficult to assess precisely how larger benthic foraminiferal diversity and abundances will develop in the near future. If indeed elevated dissolved  $\text{CO}_2$  is beneficial for foraminiferal calcification, their deterioration due to climate change and pollution may be partly mitigated and adds to the uncertainty in prediction the contribution of large benthic foraminifera on reef stability, sediment production and shallow reef biogeochemical cycling (Langer, 2008). With rates as high as  $1 \text{ kg/m}^2/\text{yr}$  (Fujita et al., 2000; Hallock, 1981; Hohenegger, 2006), larger benthic foraminifera can contribute severely to these processes locally.

Ries et al. (2009) suggest that these major differences could be related to a large range of factors such as species' ability to regulate the pH at their site of calcification, whether a species' skeleton is covered by an organic lining that separates the carbonate from direct contact to the sea water, or whether or not photosynthetic symbionts are involved. Especially the ability for pH regulation plays a major role for many calcifiers (Ries, 2011), and foraminifera are known to strongly manipulate the pH at their site of calcification and microenvironment (Glas et al., 2012; Toyofuku et al., 2017). For *Am-*

*phistegina lobifera* a strong decrease in their microenvironmental pH caused by respiration and photosynthesis has been observed, with stronger decreases under higher  $\text{atm } p\text{CO}_2$  levels (Stuhr et al., 2021). *Ammonia* sp. actively pumps out protons during calcification (Toyofuku et al., 2017), resulting in a strong decrease in the pH of the sea water in the direct microenvironment of the shell, leading to shift in the speciation of inorganic carbon. At the lowered pH relatively more inorganic carbon occurs in the form of  $\text{CO}_2$  instead of bicarbonate, which allows diffusion through the foraminiferal membrane into the cell, due to the strong gradient in concentration. Pumping out protons not only caused a decrease of pH in the microenvironment, but also a corresponding increase of the pH within the cell (de Nooijer et al., 2009), adding to the concentration gradient between sea water and the site of calcification. At the higher internal pH, the inorganic carbon speciation shifts again, causing  $\text{CO}_2$  to become  $\text{CO}_3^{2-}$ , which is then used by the cell to form  $\text{CaCO}_3$  (Toyofuku et al., 2017).

Whether this mechanism is present in all benthic foraminifera, including *A. lessonii* and *H. depressa*, remains to be investigated. Differences in the chemical composition of the shells of genera like *Ammonia*, *Amphistegina* and *Heterostegina* (Geerken et al., 2018; Raitzsch et al., 2010) suggest that they differ in the way they acquire the ions necessary for calcification (Ter Kuile and Erez, 1991; de Nooijer et al., 2014a). Finding the exact mechanisms by which they differ in their biomineralization pathway may also shed light on the difference in their response to elevated  $p\text{CO}_2$ . With substantial differences amongst foraminifera in their response to increased  $p\text{CO}_2$ , future changes in the balance between groups may be far more important for the marine inorganic carbon cycling than the current net foraminiferal calcification response to ocean acidification.

## CONCLUSION

Growth and calcification responses of large, benthic, symbiont bearing foraminifera *Heterostegina depressa* and *Amphistegina lessonii* to ocean acidification were tested by exposing them to 400, 700, 1000 and 2200 ppm of  $p\text{CO}_2$ . Calcification by these foraminifera was measured directly using the Alkalinity Anomaly Technique, counting new chambers formed and using MicroCT-scans to analyze test properties such as thickness and carbonate volume for *A. lessonii*. Carbonate production by *A. lessonii* shows an optimum at slightly elevated  $p\text{CO}_2$  levels (700 ppm), but a decrease when  $p\text{CO}_2$  levels increased further. Chamber addition rates as well as carbonate production rates by *H. depressa* follow a similar pattern.

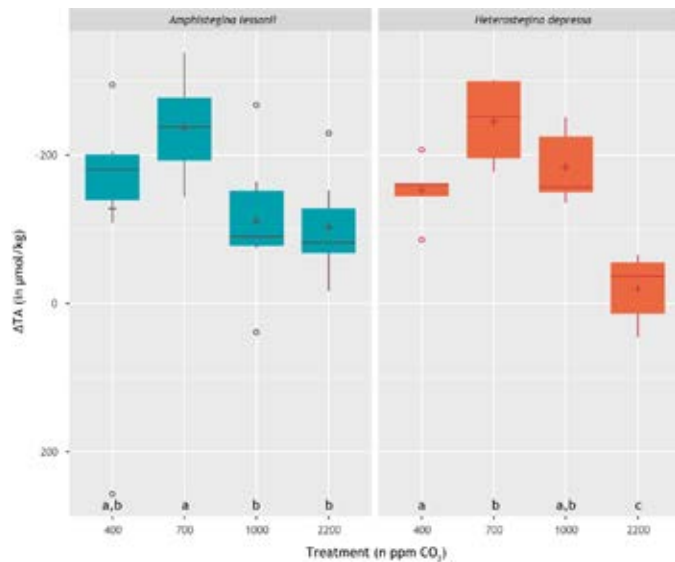


Benthic foraminifera are thus not only able to maintain calcification in moderately acidified conditions, but some species might even calcify significantly more at elevated  $p\text{CO}_2$  levels. Our results indicate that they profit from an increased amount of inorganic carbon present, before the negative effects of ocean acidification, such as higher energy costs to increase their internal pH sufficiently, tip the balance towards net negative effects.

## SUPPORTING INFORMATION

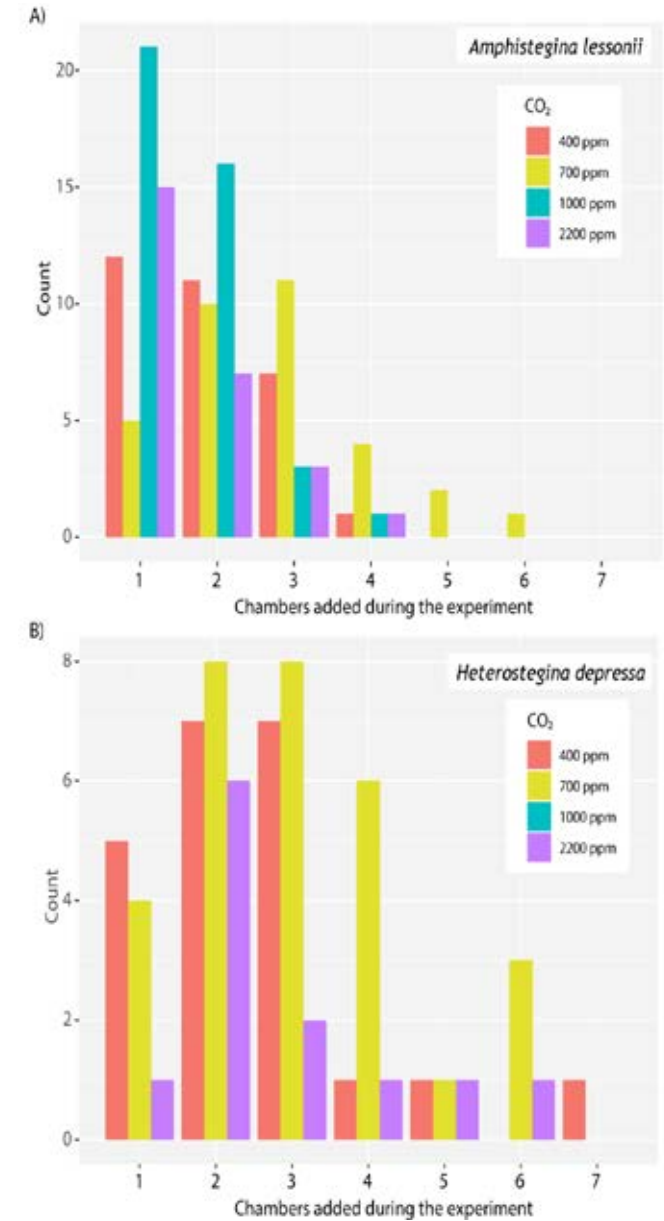
	Average changes in TA per week ( $\mu\text{mol/kg}$ ) $\pm$ 1SD	
$p\text{CO}_2$ (ppmv)	<i>A. lessonii</i>	<i>H. depressa</i>
400	-193 $\pm$ 60	-152 $\pm$ 44
700	-237 $\pm$ 66	-245 $\pm$ 58
1000	-136 $\pm$ 74	-184 $\pm$ 51
2200	-103 $\pm$ 69	-53 $\pm$ 14

**Table S1. Changes in TA for each experiment.** Negative values indicate a decrease in TA over time and hence reflect a net  $\text{CaCO}_3$  production.



**Figure S1. Relationship between  $\text{CO}_2$  and  $\Delta\text{TA}$ .** For both species the change in Total Alkalinity varies from week to week, but also between treatments. For *A. lessonii*,

TA decreased more in the two lowest  $\text{CO}_2$  treatments, for *H. depressa* the strongest decrease in TA was observed at 700 ppm. The smallest change in TA for *A. lessonii* was observed at 1000 and 2200 ppm, for *H. depressa* at 2200 ppm. Mean values are indicated by plus symbols. Letters below the boxplots indicate statistically significantly different groups ( $t$ -test,  $p$ -value  $<$  0.05). Note that the scale on the y-axis is reversed: a stronger decrease in TA indicates a more calcite produced during the experimental period.



**Figure S2. Histogram of chambers added during the experiment per treatment for the pre-stained sub-set of specimens for A) *A. lessonii* and B) *H. depressa*.** The duration of the experiment was 50 days for *A. lessonii* and 36 days for *H. depressa*. Since pre-staining with calcein did not work for the *H. depressa* specimens in the 1000 ppm treatment, no chamber counts could be determined. While peaks for all treatments were around 2-3 chambers for *H. depressa*, more specimens build a higher number of chambers in the 700ppm atm  $p\text{CO}_2$  treatment than during both higher and lower  $\text{CO}_2$  levels. A very similar pattern can be observed for *A. lessonii*.

# CHAPTER THREE

## LIGHT IMPACTS MG INCORPORATION IN THE BENTHIC FORAMINIFER *AMPHISTEGINA LESSONII*

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### ABSTRACT

The ratio of Mg to Ca in foraminiferal calcium carbonate ( $Mg/Ca_{cc}$ ) is a popular tool to reconstruct past sea water temperatures. Its application is being complicated by other environmental factors affecting the  $Mg/Ca_{cc}$ , including salinity and the ratio between sea water  $[Mg^{2+}]$  and  $[Ca^{2+}]$ . Furthermore, there is considerable intra-specimen variability in  $Mg/Ca$  in the form of alternating high- and low-concentration bands. This banding has recently been linked to diurnal cyclicality, in which bands with relatively high  $Mg/Ca$  are precipitated during night-time. Here we show that light not only impacts variability due to banding but also significantly affects average chamber  $Mg/Ca$  in the large benthic, symbiont-bearing foraminifer *Amphistegina lessonii*. These ratios are higher in foraminifera that calcify for a longer time in the dark, with a difference in  $Mg/Ca$  of 23 mmol/mol between carbonate formed completely in the dark versus completely in the light. We propose that individual timing of chamber formation and thus presence or absence of light during calcification is an important driver of inter-specimen variability in foraminiferal  $Mg/Ca$ .



## INTRODUCTION

Elements in foraminiferal calcite serve as tools to reconstruct past environments: Mg/Ca, for example, can be used to estimate paleo-temperatures (Nuernberg, 1995). However, such proxy-relationships are usually offset by other environmental variables: salinity and carbonate chemistry, for example, are known to impact Mg-incorporation (Dueñas-Bohórquez et al., 2011; Geerken et al., 2018; Hönisch et al., 2013; Lea et al., 1999), as does sea water Mg/Ca (de Nooijer et al., 2017b; Segev and Erez, 2006).

Light potentially impacts trace element incorporation in foraminiferal shells. In the planktonic species *Orbulina universa* (Holland et al., 2017; Spero et al., 2015) and *Neogloboquadrina dutertrei* (Fehrenbacher et al., 2017) bands of high element concentrations are associated to calcite formed in the dark. A potential mechanistic link between light and element incorporation was suggested by Al-Horani et al. (2003), who found light-dependent  $\text{Ca}^{2+}$ -ATPase in corals. Erez (2003) speculated that similar mechanisms could promote calcification in foraminifera, which would make element incorporation in foraminiferal calcite light dependent. This effect could be enhanced, for example by migration of planktonic species in the water column, hence affecting symbiont activity (Köhler-Rink and Kühl, 2000, 2005). Photosynthesis by these symbionts determines the pH and saturation state in the microenvironment of the foraminifera (Köhler-Rink and Kühl, 2000), as well as the oxygen levels near the foraminifera (Köhler-Rink and Kühl, 2005) which in turn, could affect the chemical speciation of trace and minor elements (e.g. S; (van Dijk et al., 2018)) and hence their uptake rates during calcification.

To quantify the relation between light and calcite chemistry, we tested the incorporation of Mg as a function of light exposure by culturing the symbiont bearing large benthic foraminifera *Amphistegina lessonii* under different durations of light exposure, while keeping all other factors constant.

## MATERIALS AND METHODS

Surface sediments were collected from the tropical reef aquarium at the Royal Burgers' Zoo (Arnhem, The Netherlands; (Ernst et al., 2011)) by scuba diving. Living specimens of *A. lessonii* >250µm were isolated and fed a solution of freeze dried *Dunaliella salina* with every exchange of culture medium. Diameters of the incubated specimens varied between ~250 and ~1000 µm, but they were distributed evenly over five groups of 10 specimens that were cultured at 6 different day/night regimes. Each of the 30 groups were placed in tissue bottles with 70ml of culture medium that were

placed in incubators with constant temperature (21°C), salinity (35.4) and light intensity (85 µmol photons/m<sup>2</sup>/s). The duration of light exposure per day varied for each treatment (4, 10, 12, 14, 20 and 24h of light per day) and lasted 25 days in total.

The culture medium consisted of modified, filtered North Atlantic surface seawater with addition of 5mg/L Calcein to stain carbonate formed during the experiment. Before and after addition of the Calcein, pH remained similar. Incorporation of this fluorescent marker is known not to impact trace metal incorporation (Dissard et al., 2009) and hence does not bias the interpretation of our results. Additionally, in four treatments (4, 10, 14 and 20h of light exposure per day) the water chemistry was artificially altered when switching on or off the light. With the light switched on, low-Ba (~4.4 µmol/mol) culture medium (ambient seawater + Calcein) was supplied. At the onset of every dark period, the culture medium was replaced by a second medium with a Ba/Ca ratio set at an elevated value of 19.0 µmol/mol (ambient seawater + Calcein + BaCl<sub>2</sub>), as a tracer for carbonate formed during the dark periods in contrast to the fluorescent dye which was incorporated during both light and dark periods. Both tracers were necessary, since using only Calcein does not allow to determine the timing of calcification. Using elevated Ba/Ca during night-time without the additional use of Calcein would result in a bias, since chambers formed completely during daytime would not be labelled and thus could not be differentiated from chambers formed before the onset of the experiment. Ba is a suitable tracer since its incorporation is mainly controlled by the concentration in the culture medium (Lea and Boyle, 1991; Lea and Spero, 1992; de Nooijer et al., 2017a). The medium with elevated Ba concentrations was produced by adding a BaCl<sub>2</sub> solution to the low-Ba seawater. The two treatments of 12hr light exposure and 24hr light exposure per day did not involve enrichment of Ba and were only used to monitor the effect of light exposure on growth rate. When replacing the culture medium with the low Ba-seawater, culture bottles were rinsed three times thoroughly with low Ba culture medium to avoid contamination with seawater with a high Ba/Ca of the preceding light phase. After termination of the experiment, all specimens were then cleaned following procedures described by van Dijk et al. (2017) and subsequently examined for new, fluorescent chambers.

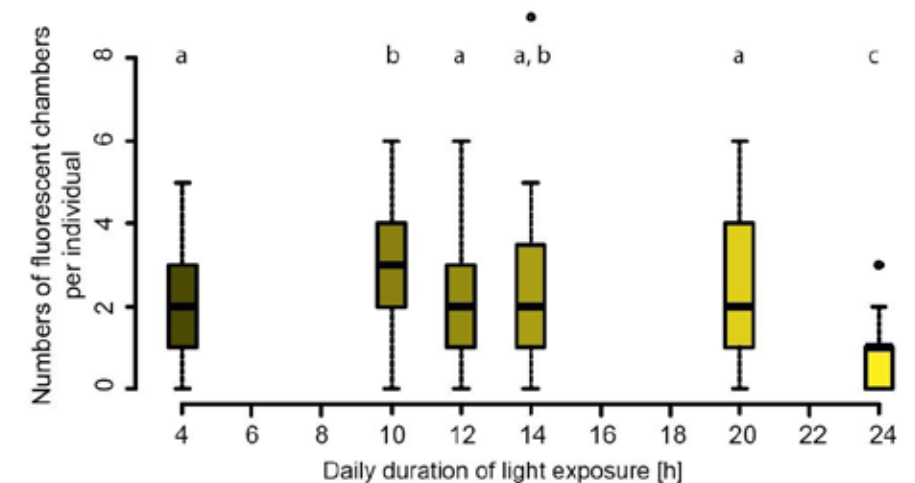
Individual chambers from 121 specimens of the enriched Ba treatments were analysed for their Ba/Ca and Mg/Ca using LA-ICP-MS. A total of 57, 57, 40 and 42 laser ablation measurements of ultimate and penultimate chambers were conducted for treatments with 4h, 10h, 14h and 20h light exposure, respectively. The first (oldest)

chamber labelled with Calcein was not analysed since the calcite of this chamber may (partly) reflect pre-experimental conditions (e.g. in case calcification would utilize a previously built internal calcium pool). In total 196 measurements were performed using 121 F and 71 F-1 chambers, if the F-1 chamber was not the first chamber labelled with Calcein. The laser system (NWR193UC, New Wave Research) at the NIOZ was used in combination with a two-volume sample cell (TV2), which allows detecting variability in El/Ca ratios within the foraminiferal chamber wall due to a short wash-out time of 1.8s (van Dijk et al., 2017b). A circular laser spot with a diameter of 80  $\mu\text{m}$  was chosen to allow measurements in the centre of the chamber. All specimens were ablated with an energy density of  $1.0 \pm 0.1 \text{ J/cm}^2$  and a repetition rate of 6Hz in a helium environment until the chamber walls were fully penetrated. A 0.7 L/m helium flow transported the resulting aerosol to an in-house-built smoothing device before entering the quadrupole ICP-MS (iCAP-Q, Thermo Fisher Scientific). Masses  $^7\text{Li}$ ,  $^{23}\text{Na}$ ,  $^{24}\text{Mg}$ ,  $^{25}\text{Mg}$ ,  $^{43}\text{Ca}$ ,  $^{44}\text{Ca}$ ,  $^{55}\text{Mn}$ ,  $^{88}\text{Sr}$ ,  $^{137}\text{Ba}$  and  $^{138}\text{Ba}$  were measured,  $^{44}\text{Ca}$  served as an internal standard. The synthetic carbonate standard MACS-3 was used for calibration, in addition carbonate standards JcP-1, JcT-1, NFHS1 (NIOZ foraminifera house standard; (Mezger et al., 2016)) as well as glass standards SRM NIST610 and NIST612 were used for monitoring data quality.

Elemental concentrations of the culture media were determined with high resolution ICP-MS (Element2, Thermo Finnigan). For determining Ba and U, the culture medium samples were diluted to 10% with a 0.14M  $\text{HNO}_3$  solution, while samples for the analyses of Mg, Ca, and Sr were diluted to 1% with 0.14M  $\text{HNO}_3$ . All samples were calibrated by matrix matched standard addition, with In as an internal standard and 1% and 10% diluted North Atlantic seawater was used for matrix matching. IAPSO standard water was used for reference. The trace metal composition of both culture media was very similar, except for the Ba content, which was served as a tracer for day- versus night calcification. The medium's Mg concentration varied only marginally (between 1.32 and 1.30 g/kg) between treatments. The relative standard deviation was less than 1% for Mg, Ba and Ca.

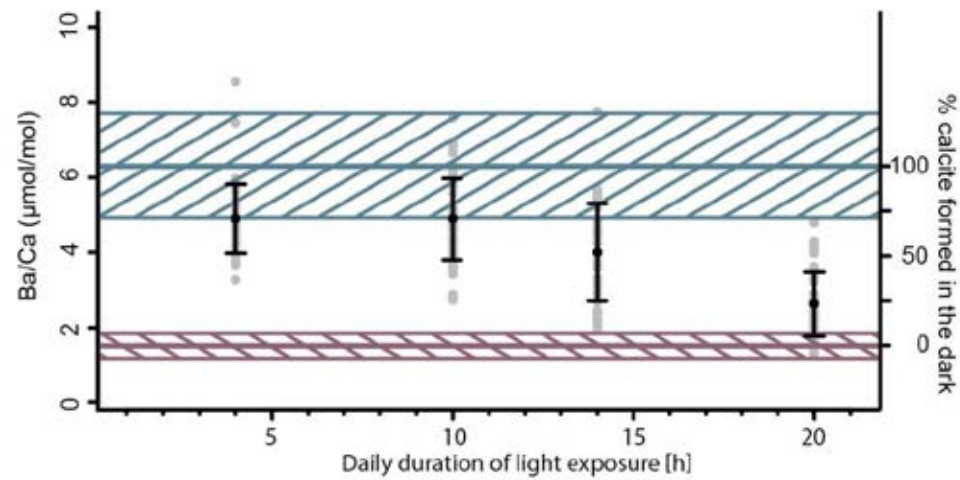
## RESULTS

Foraminifera from most treatments grew between 0 and 9 chambers per individual, with an average of 2.1 added chambers (Fig. 1). Only in the 24 hr-light group, foraminifera grew significantly fewer new chambers than in all other treatments, on average less than 1 chamber was added per individual over the duration of the entire experiment (Fig. 1). The majority of chambers formed across the different treatments were shaped and sized normally, without differences between treatments. Reproductions occurred once in both the 20h light and the 14h light treatment after 12 and 23 days, respectively.

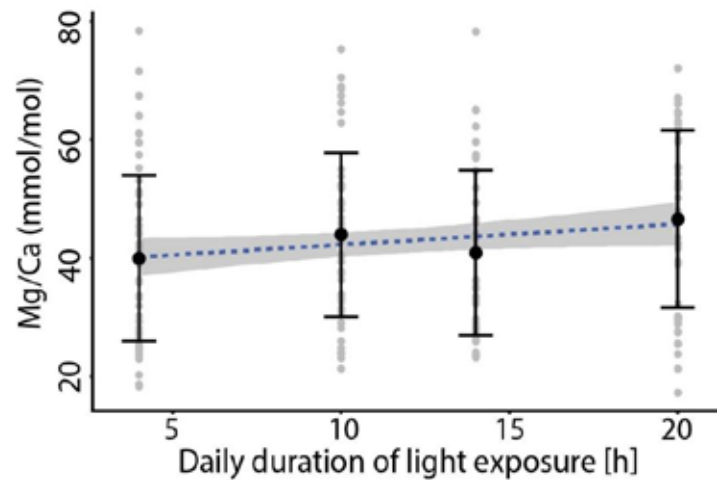


**Figure 1.** Newly formed chambers per individual for the six different treatments. Statistically significant groups ( $p > 0.05$ ) are indicated by letters. Shading indicates day length.

Values for individual chamber measured Ba/Ca vary between 1.29 and 8.53  $\mu\text{mol/mol}$ , with specimens from treatments that were exposed to high Ba for a longer time (and hence remained longer in the dark) showing higher Ba/Ca ratios (Fig. 2). Values for Mg/Ca vary between 17.3 and 78.4  $\text{mmol/mol}$  across treatments (Fig. 3), whereas Mg incorporation does not vary significantly with light duration. Average elemental composition for specimens of each treatment is reported in Table 1.



**Figure 2.** Ba/Ca ratio of the four different Ba-spiked treatments. Gray points represent single chamber measurements, black markers, and whiskers show the average  $\pm$  standard deviation of a group. The blue and red line indicate upper and lower mixing model endmembers and their uncertainties (hatched areas), based on the partition coefficient  $D_{Ba}$  and the  $Ba/Ca_{sw}$ .



**Figure 3.** Foraminiferal Mg/Ca vs. the duration of light exposure per day for the four treatments that included increased  $Ba/Ca_{sw}$  during darkness, with no significant trend between the different treatments. Black markers indicate average values  $\pm$  standard deviation. The specimens grown at 24 h light were not included since they produced very few new chambers compared to those of the other treatments.

**Table 1.** Average Elemental Composition of F-Chambers

Treatment (h light per day)	Ba/Ca ( $\mu\text{mol/mol}$ )	Mn/Ca ( $\mu\text{mol/mol}$ )	Na/Ca ( $\text{mmol/mol}$ )	Sr/Ca ( $\text{mmol/mol}$ )	Mg/Ca ( $\text{mmol/mol}$ )
4	5.00	3.44	12.66	1.75	39.95
10	5.00	1.98	11.85	1.84	44.00
14	3.99	1.87	12.13	1.82	40.93
20	2.61	2.43	12.71	1.88	46.60

Culture media used during light exposure and during darkness were very similar and varied only by the artificially increased Ba concentrations in the medium used during darkness (Table 2).

**Table 2.** Culture Media Composition

Medium	Ba ( $\mu\text{g/Kg}$ )	Mg ( $\text{mg/Kg}$ )	Ca ( $\text{mg/Kg}$ )	Sr ( $\text{mg/Kg}$ )	U ( $\text{mg/Kg}$ )
Day	5.75	1317.62	436.82	7.95	3.50
Night	25.78	1299.32	433.13	7.93	3.63

## DISCUSSION

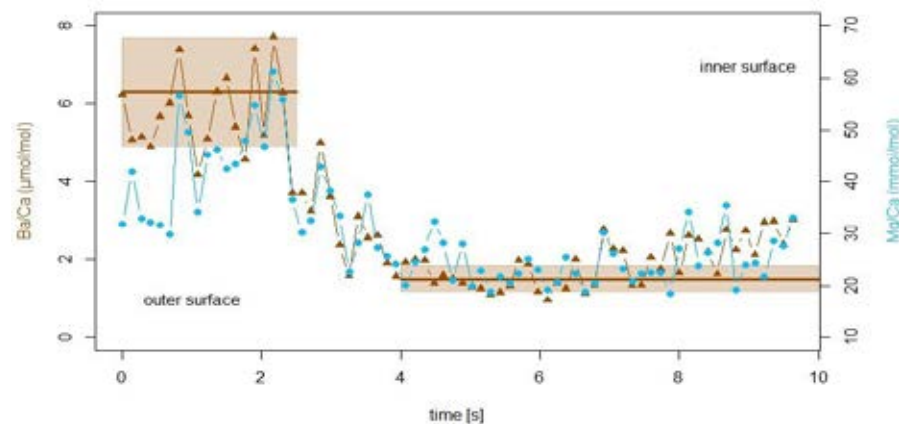
### THE RELATION BETWEEN DARKNESS AND FORAMINIFERAL GROWTH

Chamber addition rates were similar for all day lengths (Fig. 1), except for the longest day length (24h light), with less growth and for the 10h day length, where growth was slightly higher. Most conditions were thus sufficiently favourable for foraminifera to grow similarly well and hence differences in elemental composition in these groups are not likely caused by stress. The fact that the 24h of light exposure resulted in a strong

reduction in chamber addition, suggests that darkness is necessary for calcification. This is in line with results presented by Nobes et al. (2008) who observed no significant difference in growth rates of *Amphistegina* spp. cultured under three different light intensities over the course of a 96 day incubation period, though in their experiment, mortality was highest amongst specimens cultured under highest light intensity. Nobes et al. (2008) concluded that light is not a limiting factor for the growth of symbiont bearing foraminifera and that for some species, less light can result in more growth.

### DAY VERSUS NIGHT CALCIFICATION

Since foraminiferal  $Ba/Ca_{cc}$  correlates with sea water  $Ba/Ca_{sw}$  (Lea and Spero, 1992; de Nooijer et al., 2017a; Lea and Boyle, 1991) and is not significantly impacted by other environmental conditions (Hönisch et al., 2011; Lea and Spero, 1994), average test  $Ba/Ca_{cc}$  from our experiment reflect the relative amount of calcite produced during night- versus day time. Endmember concentrations, assuming a specimen added calcite only during 'day' or only during 'night', are calculated using the Ba concentrations from the culture media and the partition coefficient ( $D_{Ba}$ ) for *A. lessonii* ( $0.33 \pm 22\%$ ; de Nooijer et al., 2017a). Calcite precipitated during 'day' should hence have a  $Ba/Ca_{cc}$  of  $1.5 \mu\text{mol/mol}$  ( $\pm 22\%$ ), while calcite precipitated during 'night' should have a  $Ba/Ca_{cc}$  of  $6.3 \mu\text{mol/mol}$  ( $\pm 22\%$ ). These values are used as endmembers for our linear mixing model. Specimens exposed to sea water with elevated Ba for a longer time interval (i.e. longer 'night'), have higher average  $Ba/Ca_{cc}$  and vice versa (Fig. 2). These calculated endmember concentrations can be recognized by the plateaus of high or low Ba/Ca across the test wall in the laser ablation measurement profiles (Fig. 4).



**Figure 4.** Laser ablation ICP-MS profile of a specimen of *A. lessonii* from the 10 h light treatment.  $Ba/Ca$  ratio of the F-chamber shown in brown triangles, blue circles indicate  $Mg/Ca$  ratios. The x-axis represents duration of the measurement, while the laser was moving fully through the test wall from the outermost layer (left) toward the inside (right) of the chamber.  $Ba/Ca_{sw}$  had been enhanced during nighttime, the two plateaus in the profile represent ratios expected based on the sea water concentration and the partition coefficient ( $\pm 22\%$  uncertainty) indicated by brown lines and hatched areas. The outer part of the shell with elevated  $Ba/Ca$  formed during high  $Ba/Ca_{sw}$  exposure (night-time), while the lower plateau without elevated  $Ba/Ca$  ratios formed during low  $Ba/Ca_{sw}$  exposure (daytime).  $Mg/Ca_{sw}$  was not manipulated, yet  $Mg/Ca_{cc}$  correlates with  $Ba/Ca_{cc}$  showing that the incorporation of Mg varies between day and night-time.

Based on Ba partitioning and this observation, the  $Ba/Ca_{cc}$  of each specimen was translated into the relative amount of calcite formed during 'day' and 'night'. The distribution of Ba shows that the majority of chambers formed partially during both periods, with only few chambers showing an average  $Ba/Ca_{cc}$  that matches one of the two endmembers (Fig. 2). The distribution of single-chamber  $Ba/Ca_{cc}$  also shows that the 'night' is somewhat overrepresented in the total population. The ratio of duration of darkness versus light (day length) does not correspond 1:1 to the ratio of low-Ba-calcite to high-Ba-calcite. If the timing and duration of foraminiferal calcification would be fully random, however, high-Ba / low-Ba calcite would on average match the duration of night / day. Since this is not the case, the timing of the start of calcification and / or the duration of the chamber formation event are likely non-random. For example, only few specimens in only one treatment (20h of light exposure per day) finished forming a full chamber under low Ba conditions (i.e., during 'day' time). While a large number of specimens formed chambers that consisted of 100% high-Ba-calcite, our results suggest that foraminifera tend to precipitate the majority of their calcite during darkness.

Production of an internal calcium pool (Anderson and Faber, 1984; Erez, 2003) and its utilization during chamber formation potentially biases our results. If the internal calcium pool was derived from seawater and therefore a source of the incorporated Ba, the measured  $Ba/Ca$  may reflect the  $Ba/Ca$  of the medium prior to the calcification event. However, even in the treatment with longest light exposure (20 hours), specimens with  $Ba/Ca_{cc}$  indicating 100% daytime calcite are uncommon but are present (Fig 2). If calcification uses a previously built internal calcium pool, the longevity of this pool is much shorter (<20 hours) than previously suggested (2-3 days; (Erez, 2003)). This discrepancy may be caused by inter-species differences in the size or contribution of this pool. Calcification in *A. lessonii* may be more similar to that of other species for which

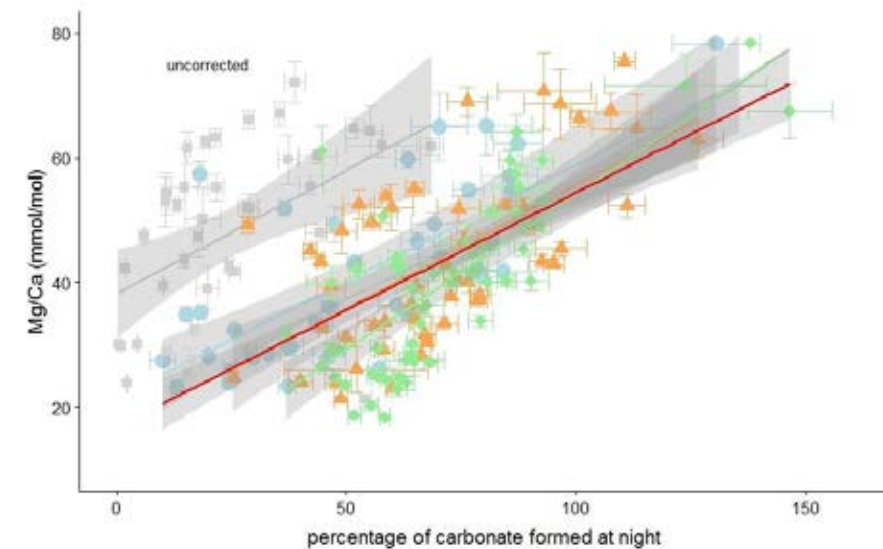


the absence of an internal calcium pool was shown (Nehrke et al., 2013). Furthermore, the observed sharp in- or decrease in the laser ablation profile of Ba/Ca in the F-chamber (Fig. 4) does not support precipitation from a pool with a given Ba/Ca. In contrast, it more likely reflects Ba-uptake during chamber formation.

Elevated seawater Ba/Ca is suspected to promote Mg incorporation in large benthic foraminifera (de Nooijer et al., 2017a). To evaluate the impact of the day-night cycle on Mg incorporation it is therefore necessary to correct for the effect of increased Ba/Ca during the night-time incubations. An increase of 1  $\mu\text{mol/mol}$  in  $\text{Ba/Ca}_{\text{cc}}$  caused by increased  $\text{Ba/Ca}_{\text{sw}}$  is associated with an increase of 3.1  $\text{mmol/mol}$   $\text{Mg/Ca}_{\text{cc}}$  in *A. lessonii* under otherwise constant conditions (de Nooijer et al., 2017a). A similar relationship between  $\text{Mg/Ca}_{\text{cc}}$  and  $\text{Ba/Ca}_{\text{cc}}$  has been found for some planktonic foraminifera (Hathorne et al., 2009; Hori et al., 2018) but not for others (Hori et al., 2018), suggesting species-specific controls on Ba- and Mg-incorporation. Even though no causal explanation or potential mechanism for this effect is presently known, we corrected our data for this observed co-variation. To this end, the correlation between elevated  $\text{Ba/Ca}_{\text{cc}}$  and  $\text{Mg/Ca}_{\text{cc}}$  presented in de Nooijer et al. (2017a) was first corrected to the correct Mg concentrations based on the experimental temperatures used. The difference in  $\text{Mg/Ca}$  [ $\text{mmol/mol}$ ] due to a 4°C temperature offset was calculated using a previously published  $\text{Mg/Ca}$ -temperature calibration for this species (van Dijk et al., 2018). In contrast, the Ba concentrations were not adjusted, since temperature is reported to have no effect on foraminiferal  $\text{Ba/Ca}_{\text{cc}}$  (Hönisch et al., 2011; Lea and Spero, 1994). The resulting linear regression of  $\text{Mg/Ca}_{\text{cc}}$  [ $\text{mmol/mol}$ ] =  $3.1 * \text{Ba/Ca}_{\text{cc}}$  [ $\mu\text{mol/mol}$ ] - 10.36 (at 21°C) was applied to correct the potential impact of Ba on the Mg concentrations in this study. The temperature corrected regression was subtracted from the observed relationship between Ba/Ca and Mg/Ca ( $\text{Mg/Ca}_{\text{cc}}$  [ $\text{mmol/mol}$ ] =  $7.84 * \text{Ba/Ca}_{\text{cc}}$  [ $\mu\text{mol/mol}$ ] + 5.03), to result in a final, Ba-effect corrected regression of  $\text{Mg/Ca}_{\text{cc}}$  [ $\text{mmol/mol}$ ] =  $4.74 * \text{Ba/Ca}_{\text{cc}}$  [ $\mu\text{mol/mol}$ ] + 10.39. Explaining the entire observed correlation with Ba addition only would imply a 2.5 times higher sensitivity of Mg to Ba addition, which is very unlikely as we used the same species, same experimental set up and analytical approach. Overall, the impact of Ba (in the micro mole range) on Mg (in the millimol range) is likely very limited because of their very different relative contributions to the carbonate.

## IMPACT OF DAY LENGTH ON ELEMENT INCORPORATION

Foraminiferal Mg/Ca is significantly correlated with the calculated portion of carbonate formed at 'night' (Fig. 5). Three treatments show very similar relationships, however, the 20h light exposure group is offset from the others, although the slope of the regression with percentage of carbonate formed in the dark is similar (Fig. 5, Table 3(a)).

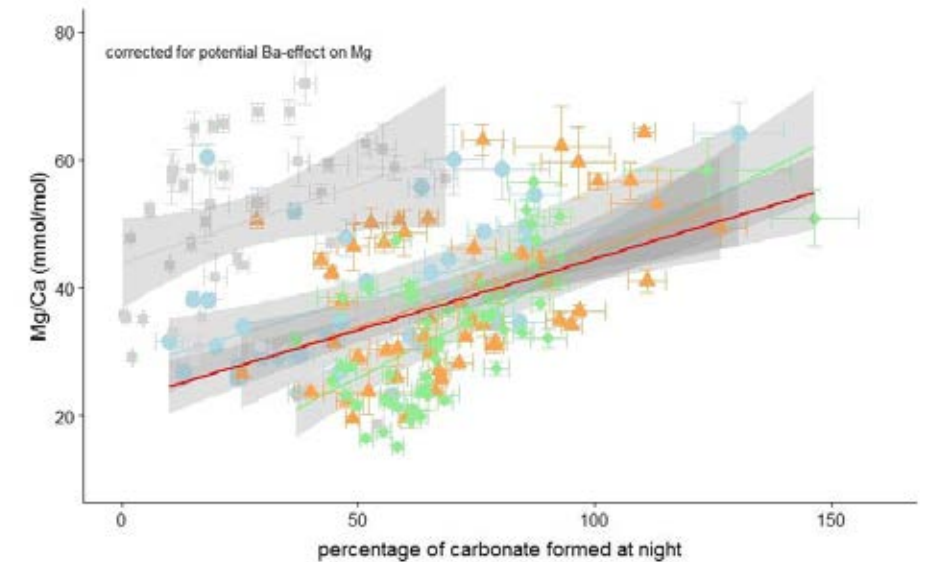


**Figure 5.** Uncorrected foraminiferal Mg/Ca versus the percentage of carbonate formed during darkness. Elevated Ba concentrations in the culture medium at night has been used to calculate the percentage of carbonate formed during darkness (Figure 2). Individual measurements of specimens from the 4, 10, 14, and 20 h light per day treatment are indicated by green diamonds, orange triangles, blue circles, and gray squares, respectively. Horizontal and vertical error bars represent  $\pm$  SEM on the Ba/Ca and Mg/Ca, respectively. Regressions can be found in Table 3a. Percentages over 100% are possible due to the uncertainty in the  $\text{Ba/Ca}_{\text{cc}}$ - $\text{Ba/Ca}_{\text{sw}}$  regression (de Nooijer et al., 2017a).

**Table 3.** Relationship between Mg/Ca [mmol/mol] and % of carbonate formed at night

treatment (hr light per day)	Slope	Intercept	adjusted R <sup>2</sup>	p-value
(a) uncorrected				
4	0.50	4	0.57	p<0.001
10	0.40	16	0.41	p<0.001
14	0.38	21	0.52	p<0.001
20	0.46	36	0.30	p<0.001
4,10 and 14 combined				p<0.001
(b) corrected for potential Ba effect				
4	0.34	7	0.44	p<0.001
10	0.24	22	0.20	p<0.001
14	0.22	27	0.27	p<0.001
20	0.31	42	0.14	p<0.01
4, 10 and 14 combined	0.23	23	0.21	p<0.001

The absence of darkness clearly reduced chamber addition rates and therefore short (i.e., 4 hr) nights may have had a minor effect on calcification. We hypothesize that although chamber addition was not hampered (Fig. 1), the 4-hour night duration altered Mg incorporation in these foraminifera and offset the Mg/Ca-%calcite formed at night relationship (Fig. 5). Even after correcting for a potential effect of elevated Ba on Mg incorporation, Mg/Ca in the foraminiferal calcite is significantly correlated with the percentage of calcite that was formed during darkness (Fig. 6, Table 3(b)).

**Figure 6.** Foraminiferal Mg/Ca versus the percentage of carbonate formed during darkness corrected for a potential impact of the Ba-label on the Mg-content. Individual measurements of specimens from the 4, 10, 14, and 20 h light per day treatment are indicated by green diamonds, orange triangles, blue circles, and grey squares, respectively. Horizontal and vertical error bars represent  $\pm$  SEM on the Ba/Ca and Mg/Ca, respectively. Regressions can be found in Table 3a. Percentages over 100% are possible due to the uncertainty in the  $Ba/Ca_{cc}-Ba/Ca_{sw}$  regression (de Nooijer et al., 2017a).

Correlating the Mg/Ca to the portion of carbonate formed during ‘day’ versus the portion formed at ‘night’ shows a relationship that, after correcting for a potential Ba-effect on Mg incorporation, can be described with the following equation:

$$\text{Mg/Ca [mmol/mol]} = 0.23 * \% \text{dark\_calcite} + 22.5. \quad (1)$$

Therefore, the calcite endmember corresponding to only daylight (%dark\_calcite=0) would have a Mg/Ca<sub>cc</sub> of 22.5 mmol/mol, while calcite formed completely during darkness (%dark\_calcite=100) has a much higher Mg/Ca<sub>cc</sub> of 45.5 mmol/mol (Fig. 6). The slope in Mg/Ca as a function of %dark\_calcite is therefore an increase of about 2.3 mmol/mol Mg/Ca per 10% decrease of light exposure duration during calcification. Translating this to temperature reconstructions (van Dijk et al., 2018), implies an effect of  $\Delta +1.36^\circ\text{C}$  in *A. lessonii* per 10% increase in light exposure during calcification. Clearly

such changes could be of major importance, especially when investigating temperatures at high latitudes where differences in day lengths are substantial, assuming that high latitude inhabitants respond similarly to day/night cyclicality as *A. lessonii*. For *A. lessonii* the average Mg/Ca of the four different day length treatment is not significantly different (Fig. 3), therefore temperature reconstructions do not have to be corrected for a latitudinal effect on day length and thus foraminiferal Mg/Ca. Due to potential species-specific effects, this remains to be tested for other benthic as well as planktonic species.

Although the effect of light exposure on magnesium incorporation is evident, the underlying mechanism is not. Photosynthesis changes the pH and saturation state in the microenvironment of the foraminifera, pH can change from 8.03 under dark conditions to 8.12 during light exposure in *A. lobifera* (Köhler-Rink and Köhl, 2000). An increase of 0.1 pH unit has been shown by Lea et al. (1999) to lead to a decrease of 6% in the Mg/Ca in the tests of planktonic foraminifera such as *Orbulina universa* and *Globigerina bulloides* although no correlation between the Mg content of the tests and light or dark exposure was found. Dependency of Mg incorporation on the inorganic carbon system has also been shown for large benthic foraminifera such as *Operculina ammonoides* (Evans et al., 2018), but no significant correlation between  $p\text{CO}_2$  (co-varying with pH) and Mg/Ca has been found for large benthic foraminifera *Amphistegina gibbosa* (van Dijk et al., 2017b). The latter suggests that it is unlikely that pH explains the here observed changes in Mg/Ca as a function of light exposure in. Although the calcification process may differ between (and within) planktonic and benthic genera, the sensitivity of planktonic Mg/Ca to seawater pH may indicate that the calcite chemistry of planktonic species may be even more vulnerable to the effect of day/night changes.

The large variability of elemental ratios between as well as within species, that is often observed in culture studies with controlled, constant environmental factors (e.g. Hathorne et al., 2009; de Nooijer et al., 2014; Geerken et al., 2018) appears to be at least partly caused by differences in the exposure of the different specimens to light during chamber formation. These differences, in turn, could be caused by differences in the timing of foraminiferal calcification such as duration or time of calcification onset. Segev & Erez (2006) as well as Hathorne et al. (2009) suggested that a large part of variation observed in foraminiferal Mg/Ca could be caused by physiological processes in addition to environmental impacts. Similar changes in  $\text{Mg}/\text{Ca}_{\text{cc}}$  occur in both symbiont bearing and -barren species, indicating that symbionts are not the driving factor for these variations.

## CONCLUSION

In addition to previously known intra shell variations in Mg/Ca that correlated with a dark-light cycle (e.g. Spero et al., 2015; Fehrenbacher et al., 2017), here we show that also average chamber Mg/Ca values are strongly affected by light exposure. Though a population's average Mg/Ca does not appear to be influenced by different light exposure caused by natural factors such as seasons as well as latitude, since the average of Mg/Ca in all four treatments with daily light exposure ranging from 4 to 20h is very similar. Even though there are substantial effects on the individual foraminifera, this should not introduce a bias during application as a paleothermometer, when large numbers of specimens are analysed. Individual timing of chamber formation, duration and time of beginning, though have a strong impact on an individual chamber's Mg/Ca. An *A. lessonii* chamber completely formed in the dark is on average 23 mmol/mol increased in Mg/Ca compared to a chamber completely formed during light exposure. This factor explains a large part of inter-specimen variation previously observed but unexplained in culture studies (e.g. Geerken et al., 2018; de Nooijer et al., 2014).

## DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

## AUTHOR CONTRIBUTIONS

All authors designed the study. LD and LdN carried out the sampling and laboratory work. LD performed the measurements, carried out the data analysis, and drafted the manuscript. LdN and G-JR critically revised the manuscript. All authors approved the final version of the manuscript for publication.

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# CHAPTER FOUR

## TEMPERATURE IMPACT ON MAGNESIUM ISOTOPE FRACTIONATION IN CULTURED FORAMINIFERA

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### ABSTRACT

Element incorporation in shell calcite precipitated by foraminifera reflects the chemical and physical properties of the seawater the foraminifera lived in and can therefore be used to reconstruct paleo environmental conditions. One of the most prominent proxies for past seawater temperature is Mg/Ca of foraminiferal calcite. Still, in addition to seawater temperature, also biomineralization processes impact foraminiferal Mg/Ca values. As the impact of biomineralization plays a major role and is not necessarily constant, it is imperative to identify the mechanism by which Mg is incorporated and thereby understand how temperature influences Mg incorporation. Biomineralization is discriminating against Mg to different degrees and hence investigating the fractionation of Mg isotopes at different temperatures and for species with contrasting calcification pathways can be used to better understand the pathway of Mg during biomineralization. Overall, we observe that foraminifera with higher Mg content have  $\delta^{26}\text{Mg}$  values closer to those of seawater. Moreover, controlled temperature culture experiments show that parallel to an increase in Mg/Ca,  $\delta^{26}\text{Mg}$  in the tests of large benthic foraminifer *Amphistegina lessonii* decreases when sea water temperatures increase. This negative correlation between shell Mg/Ca and  $\delta^{26}\text{Mg}$  suggests a two-step control on the incorporation of Mg during biomineralization. Using a simple model, we can explain both trends as a result of a stable Mg pool, which is only little fractionated

with respect to sea water and a temperature dependent Mg pool which shows a higher fractionation with respect to sea water during biomineralization. The stable, not much fractionated pool is relatively large in high Mg foraminifera, whereas for the low Mg foraminifera the transport of Mg over a cell membrane probably results in the observed inverse correlation. Here we present a model using the Mg isotope fractionation we established for *A. lessonii* to explain the general trends for both high- and low-Mg/Ca foraminifera. A process-based understanding remains crucial a robust interpretation of foraminiferal Mg-isotopes.

## INTRODUCTION

Past climates and environments can be reconstructed using foraminiferal shell chemistry. The fractionation of many stable isotopes and incorporation of many elements (i.e., proxies) are known to be dependent on seawater chemistry and/or physical parameters. Temperature, for example, influences the foraminiferal calcite's oxygen stable isotope ratio ( $\delta^{18}\text{O}_{\text{calcite}}$ ; McCrea, 1950; Urey et al., 1951) and salinity affects the Na/Ca of foraminiferal calcite (Allen et al., 2016; Bertlich et al., 2018; Mezger et al., 2016; Wit et al., 2013). With appropriate calibrations, these chemical imprints can be used to reconstruct seawater salinity and temperature and thereby allow inferring regional or global (changes in) climate. The Mg/Ca value of foraminiferal calcite reflects sea water temperature (Nürnberg et al., 1996) and can therefore be applied to fossil foraminifera to reconstruct past bottom water (Lear et al., 2000) and sea surface (Barker et al., 2005) temperatures. The exact mechanisms behind this relation between Mg content and temperature remains elusive. Even for inorganic carbonates, for which the effect of temperature on Mg/Ca has been comprehensively studied by controlled precipitation experiments (e.g. Mucci, 1987), no real consensus has been made so far. Increased precipitation rate at higher temperatures has been proposed to explain an increase in Mg incorporation at higher temperatures (Chilingar, 1962), but this has been also disputed by several studies (Mucci et al., 1985; Mucci and Morse, 1983).

Even though the fundamentals behind Mg incorporation as a function of temperature in carbonates are illusive, foraminiferal Mg/Ca remains a popular proxy for temperature reconstructions (Anand et al., 2003; Nürnberg et al., 1996). There are, however, other complications that must be considered when applying this proxy. It has been shown that beside the major impact of temperature, also other variables like salinity and pH can have a minor, but measurable effect on Mg incorporation (Allen et al., 2016; Geerken et al., 2018; Gray et al., 2018; Kisakürek et al., 2008). Furthermore, foraminiferal

Mg/Ca in particular is highly species-dependent, leading to species-specific Mg/Ca-temperature calibrations (Toyofuku et al., 2011). What is common for foraminifera, however, is that they incorporate (much) less Mg than is expected from inorganic (i.e., non-biological) precipitation of calcite from seawater (Bentov and Erez, 2006). The biomineralization mechanism responsible for the formation of foraminiferal chambers apparently prevents incorporation of much of the Mg present in seawater either by (active) removal or exclusion of Mg during the calcification process (Bentov and Erez, 2006; Erez, 2003; de Nooijer et al., 2014a). This strong control of foraminifera on Mg uptake or removal results in a test composition 10- to 100-fold lower in  $\text{Mg}/\text{Ca}_{\text{calcite}}$  than expected from inorganically precipitated calcite (Segev and Erez, 2006; Zeebe and Sanyal, 2002). To illuminate the pathway of Mg during calcification and the effect of temperature on this process, we can investigate the magnesium isotopic signature of these foraminifera ( $\delta^{26}\text{Mg}$ ), since selective removal or uptake of Mg would likely change Mg fractionation.

Previous measurements on foraminiferal  $\delta^{26}\text{Mg}$  include mainly small planktonic and benthic hyaline species as well as porcelaneous species (Chang et al., 2004; Maeda et al., 2019; Pogge von Strandmann, 2008; Wombacher et al., 2011). Their observations suggest that foraminiferal species with low Mg incorporation also have a relatively depleted Mg isotopic composition compared to other (biogenic) carbonates. Porcelaneous species, with a higher Mg content, have a lower magnitude of fractionation, leading to a less depleted Mg isotopic composition which is closer to other organisms e.g. corals calcite (Pogge von Strandmann, 2008; Saulnier et al., 2012). Estimates of non-biological fractionation vary, but are in the order of -1 to -3.88‰ (Galy et al., 2002; Mavromatis et al., 2013; Saulnier et al., 2012), and could be a function of precipitation rate (Mavromatis et al., 2013). The additional fractionation by hyaline and porcelaneous foraminiferal species is -3.2 to 5.5‰ (summarized in Pogge von Strandmann, 2008). The offset in the magnitude of Mg fractionation between hyaline and porcelaneous foraminifera supports the concept that these groups precipitate their shells using contrasting calcification pathways. The largest fractionation has been found for species with a low Mg content (Wombacher et al., 2011), which may reflect the cellular mechanism that they employ to create a supersaturated fluid with considerably less Mg than that of seawater. This observation supports the hypothesis that occasional transport of Mg ions by transmembrane calcium transport (Nehrke et al., 2013; de Nooijer et al., 2017b) is an important source for the Mg that is incorporated into the foraminiferal shell wall of hyaline foraminifera, since cross-membrane ion transport favours lighter isotopes over heavy ones. However, the relation between Mg content and  $\delta^{26}\text{Mg}$  needs to be studied in more detail to provide insights into processes involved in Mg uptake or removal during

calcification. For this purpose, especially larger benthic foraminifera are of interest, since this group includes species which have hyaline shells but intermediate to high Mg content. This group has only been studied once before (Maeda et al., 2019) but could provide vital insights into the hyaline calcification pathway.

If the amount and isotopic composition of Mg incorporated both are determined by the same process within the biomineralization mechanism, the  $\delta^{26}\text{Mg}$  will also reflect temperature and hence may be used as a palaeoceanographic temperature proxy. First results show a negative relation between culture temperature and foraminiferal shell  $\delta^{26}\text{Mg}$  (Maeda et al., 2019), which is opposite to the relationship observed in inorganically precipitated calcite (Galy et al., 2002; Li et al., 2012), though earlier studies showed no significant impact of temperature on foraminiferal  $\delta^{26}\text{Mg}$  (Pogge von Strandmann, 2008; Wombacher et al., 2011), or inorganically precipitated calcite (Saulnier et al., 2012). If foraminiferal  $\delta^{26}\text{Mg}$  is independent of temperature but at the same time correlated to the average Mg/Ca of a species, it may constrain Mg/Ca-based temperature reconstructions based on species for which no modern-day calibration is available. This is, for example, a challenge when relying on extinct species (i.e. when reconstructing climates on longer timescales), though variability in sea water Mg isotope composition over geological time scales would have to be taken into account. To investigate the link between temperature and foraminiferal Mg/Ca and  $\delta^{26}\text{Mg}$ , we cultured the large benthic species *Amphistegina lessonii* at a range of temperatures (18 to 26°C) and investigated the relationship between  $\delta^{26}\text{Mg}$  and Mg/Ca in a range of benthic foraminiferal species.

## MATERIALS AND METHODS

### FORAMINIFERAL SAMPLES

In this study we analysed specimens of *Amphistegina lessonii* grown under a range of controlled temperature conditions, as well as larger benthic foraminifera collected from an Indo-Pacific reef aquarium. Several species of foraminifera were selected from surface sediment samples of coral debris from the tropical reef aquarium at Royal Burgers' Zoo in Arnhem, The Netherlands which is known for a large diversity in benthic foraminifera (Ernst et al., 2011). Specimens of *A. lessonii*, *Heterostegina depressa*, *Sorites orbitolis*, (*Spiroloculina angluata*, *Spiroloculina communis*, *Quinqueloculina pseudoreticulina* and *Miliolinella labiosa* were selected from the sediment to investigate the relation between Mg/Ca and  $\delta^{26}\text{Mg}$ . Due to low numbers, specimens of *Spiroloculina angluata* and *Spiroloculina communis* were combined into the group '*Spiroloculina* spp.'

For the temperature-controlled culture experiment, living specimens of the benthic, symbiont bearing foraminifera *Amphistegina lessonii* were transferred from sediment to Petri dishes and kept at constant temperature (21°C) and salinity (32) using (0.2  $\mu\text{m}$ ) filtered but otherwise unmodified North Atlantic Sea water as a culture medium. Specimens were fed with a solution of freeze-dried *Dunaliella salina* in sea water every week, and light was provided 12h per day. These specimens were used as the parental generation for the culture experiment.

### CULTURE SET-UP

Asexual reproductions events resulted in a large number (>30) of genetically identical offspring called a 'clone group'. Two days after the reproduction events, when the juveniles had developed two to three chambers, all juveniles from the same clone group were collected and divided into three subgroups. This procedure is required to confidentially exclude genetic variability as a potential cause for any potential differences observed in the outcome of the experiment (de Nooijer et al., 2014b). Each subgroup was transferred into set-ups with one of three temperature settings (18, 21 and 26°C), while all other factors (such as food availability and salinity) are kept constant, identically to the culture conditions of the parental generation. Salinity was measured weekly and was found to be constant at 32, DIC was monitored weekly and was found to be  $2153 \pm 138 \mu\text{mol/L}$  (mean  $\pm$  SD) across all treatments, temperatures remained within  $\pm 0.1^\circ\text{C}$  of the target values and to simulate a natural daylight cycle, light was switched on 12h per day. Cultures were maintained for 8 weeks to allow for sufficient growth, and at the end of the experiment foraminifera had a final diameter of at least 200 $\mu\text{m}$ . Due to the very small proportion of carbonate in the initial 2-3 chambers of the foraminifera present at onset of the experiment compared to the total amount of carbonate present at the end of the experiment, it can be assumed that the impact of the initial chambers' composition on the overall shell composition is negligible.

All specimens were then cleaned using an adapted version of the 'Barker protocol' (Barker et al., 2003). In short, foraminifera were cleaned using an oxidizing step in which organics were removed with a  $\text{H}_2\text{O}_2$  solution (0.5 ml 30% w/v  $\text{H}_2\text{O}_2$  in 49.5 ml 0.1 M  $\text{NH}_4\text{OH}$ ), and consequently, after gentle ultrasonication, rinsed with ultrapure water and dried in a laminar flow cabinet and dissolved in preparation for isotope measurements. For each Mg isotope analysis about 60 $\mu\text{g}$  of material, roughly 6 specimens per measurement, was dissolved.



## MAGNESIUM ISOTOPE ANALYSES

### MAGNESIUM ISOTOPE ANALYSES AT MARUM

Samples from the culture experiment and the aquarium were measured on two different occasions. Samples from the Indo-Pacific aquarium at Burger's Zoo and the inhouse Mg isotope standard NIOZ-Mg, consisting of 1000 µg/g Mg in 5% HNO<sub>3</sub> were measured in the Isotope Geochemistry Laboratory at MARUM, University of Bremen (Germany).

At MARUM, we followed the method of Wilckens et al. (2019) and adapted it to the sample matrix of foraminifera by using a one-step separation instead of a two-step separation. Approximately 1.5 µg Mg was loaded onto Bio-Rad BIO-spin® columns filled with 1 ml of the Bio-Rad cation exchange resin AG 50W X8 (200-400 mesh). The purification and elution of Mg was done with 0.8M HCl. The separation is based on published distribution coefficients for the resin AG 50W X8, 200-400 mesh (after Strelow, 1960; Strelow et al., 1965). The reliability of each chemical session was checked with a procedural blank and reference materials. Mg isotope measurements were performed on a ThermoFisher Scientific Neptune Plus MC-ICP-MS equipped with a stable introduction system (SIS) and a high-efficiency x-cone in low and medium resolution as described in (Vogl et al., 2020). The purified samples were dissolved in 2% HNO<sub>3</sub> and adjusted to 200 ng/g Mg. The measurements were done in the standard-sample bracketing method using a pure Mg ICP standard (Alfa Aesar Magnesium plasma standard solution, Specpure) as the bracketing standard. The evaluation of single measurement sequences and the conversion of the measured Mg isotope ratios into the DSM-3 scale, the international reference materials DSM-3 and Cambridge-I (Cam-I) were analysed in every sequence.

The uncertainty of the samples is reported as two standard deviations (2sd). The instrumental precision and internal long-term repeatability for δ<sup>26</sup>Mg was ± 0.07‰ and for δ<sup>25</sup>Mg was ± 0.04‰ (2sd, n=5), obtained by the repeated analysis of the reference material Cambridge-I (Cam-I). δ<sup>26</sup>Mg values for an internal seawater standard (bottom seawater SuSu Knolls; δ<sup>26</sup>Mg = -0.82 ± 0.08‰; δ<sup>25</sup>Mg = 0.43 ± 0.07‰; 2sd, n=3), JCP-1 (δ<sup>26</sup>Mg = -1.99 ± 0.04‰; δ<sup>25</sup>Mg = -1.04 ± 0.08‰; 2sd, n=4) and Cam-I (δ<sup>26</sup>Mg = -2.55 ± 0.05‰; δ<sup>25</sup>Mg = -0.44 ± 0.06‰; 2sd, n=6) are within analytical uncertainty in agreement with literature values, calculated from published values for seawater by Foster et al. (2010; δ<sup>26</sup>Mg = -0.82 ± 0.06‰; δ<sup>25</sup>Mg = -0.43‰; 2sd, n=26), for JCP-1 by (Wombacher et al., 2011; δ<sup>26</sup>Mg = -2.01; δ<sup>25</sup>Mg = -1.05‰) and for Cam-I by Pogge von Strandmann et al. (2011; -2.62 ± 0.04; 2sd, n=43).

### MAGNESIUM ISOTOPE ANALYSES AT NIOZ

Samples of *Amphistegina lessonii* from the temperature-controlled culture experiment were measured at the Royal NIOZ, using triplicates, each consisting of 60µg foraminiferal carbonate. Mg from the samples was purified by passing them through cation exchange columns with an aspect ratio of ~14.2, containing 900 µL AG-50W X12 (wet) resin (Bio-Rad) following a miniaturized procedure of Pogge Von Strandmann (2008). Mg isotope ratios were determined using a ThermoFinnigan Neptune Plus multicollector ICP-MS equipped with a Scott-double pass cyclonic spraychamber (SIS) equipped with a PFA-ST nebulizer using a 50 µL/min uptake capillary (ESI). For higher sensitivity, a Jet-sample cone was used in combination with a H-skimmer cone. The instrument was tuned daily for optimum signal stability with a typical sensitivity of ~70V/ppm (typical measurement solutions contained 100 ng/g Mg dissolved in 2% HNO<sub>3</sub> made from 2 times sub-boiling distilled HNO<sub>3</sub>) and a background lower than 6 mV (<sup>24</sup>Mg) using Faraday collectors equipped with amplifiers using 10<sup>11</sup> Ω resistors. Initial measurements included Ca and Na for monitoring possible matrix interferences prior the Mg isotopic analysis, but this was omitted after several analytical runs where no Ca or Na was detected in the purified samples. North Atlantic 0.2 µm filtered seawater (NASW) was included as a long-term procedure standard and as reference material in every analytical session. Samples and reference materials were measured using sample-standard bracketing against an inhouse ICP Mg (NIOZ-Mg) standard (Alpha Aesar). All solutions were matched within 10% of the Mg concentration. Values were converted to the DSM-3 scale using the NIOZ-Mg house standard, which was found to have a δ<sup>25</sup>Mg of -0.77‰ (±0.04‰, 2sd) and δ<sup>26</sup>Mg of -1.48‰ (±0.06‰, 2sd) relative to standard DSM-3. This was measured at MARUM (2.3.1). NASW was found to have δ<sup>25</sup>Mg of -0.44‰ (±0.02‰, 2sd) and δ<sup>26</sup>Mg of -0.85‰ (±0.04‰, 2sd) relative to standard DSM-3, which is very close to values reported by (Pogge von Strandmann, 2008; δ<sup>25</sup>Mg = -0.44‰ (±0.08‰, 2sd), δ<sup>26</sup>Mg = -0.83‰ (±0.09‰, 2sd)) and (Young and Galy, 2004; δ<sup>25</sup>Mg = -0.42‰ (±0.08‰, 2sd), δ<sup>26</sup>Mg = -0.82‰ (±0.04‰, 2sd)). Measured isotope ratios were converted to δ<sup>25</sup>Mg and δ<sup>26</sup>Mg values using the following equation

$$\delta^x Mg (‰) = \left( \frac{{}^x Mg / {}^{24} Mg_{sample}}{{}^x Mg / {}^{24} Mg_{standard}} - 1 \right) \cdot 1000$$

where *x* is either 25 or 26.

Standard measurements and analytical details are summarized in Tables 1 and 2.



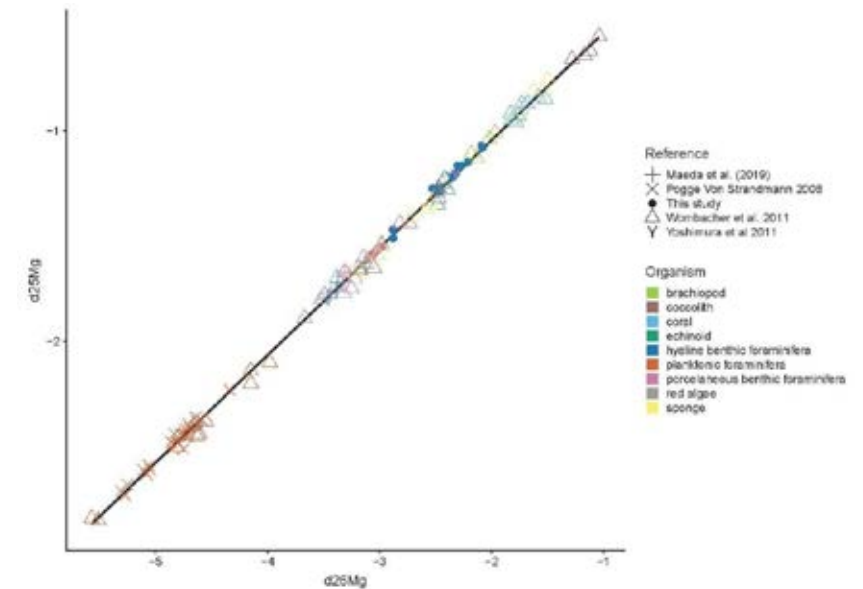
**Table 1.** Summary of standard measurements at NIOZ

	$\delta^{25}\text{Mg}$ ‰	2SD	$\delta^{26}\text{Mg}$ ‰	2SD
NASW corrected for DSM3 NIOZ	-0.44	0.02	-0.85	0.04
NASW Pogge von Strandmann et al. 2011 (n=11)	-0.44	0.08	-0.83	0.09
NASW Young and Galy 2004 (n=6)	-0.42	0.08	-0.82	0.04

**Table 2.** Summary of analytical details used during measurements at NIOZ.

Parameter	Setting
rf Power	1220 Watt
Acceleration Voltage	10 kV
Sample gas	Argon
Sample Gas Flow rate (L/min)*	0.950
Auxiliary gas flow rate (L/min)*	0.95
Coolant gas flow rate (L/min)	15.00
Fore vacuum pressure (mbar)	< 6E <sup>-4</sup>
High Vacuum pressure (mbar)	< 1E <sup>-7</sup>
Analyzer pressure (mbar)	< 8E <sup>-9</sup>
Sample uptake rate	~60 $\mu\text{L}/\text{min}$
Resolution	Low (R(5%-95%) = 1800)
Typical <sup>24</sup> Mg sensitivity	~73 V/ppm
Blank signal	0.006V for 2% HNO <sub>3</sub>
Sampling time	45 repetitions of 4s
Baseline	30 times 1.05s
Background time	One repetition of 30s. (defocused: Baseline + Background)
Cone combination	Jet + H
Multi collector	Low mass side (L1): 24 Mg Center cup (C): 25 Mg High mass side (H1): 26 Mg

Comparing these measured and corrected values with  $\delta^{25}\text{Mg}$  and  $\delta^{26}\text{Mg}$  values of other marine carbonates (Fig. 1), all  $\delta^{25}\text{Mg}$  and  $\delta^{26}\text{Mg}$  follow the same linear relationship, the terrestrial line, proving the internal consistency of this new data set.



**Figure 1.** The relationship between  $\delta^{25}\text{Mg}$  and  $\delta^{26}\text{Mg}$  values in our dataset combined with published data from other biogenic carbonates (Maeda et al., 2019; Pogge von Strandmann, 2008; Wombacher et al., 2011; Yoshimura et al., 2011) can be described as  $\delta^{25}\text{Mg} = 0.510 * \delta^{26}\text{Mg} - 0.024$  (p value < 2e-16; R<sup>2</sup> = 0.998).

Potential CN interferences, while technically possible due to contamination by carbon from the atmosphere or from the resin used are highly unlikely. Standard, blanks and sample measurements would be affected by atmospheric carbon alike, considering the stability of atmospheric CO<sub>2</sub> concentrations on such short time scales as the measurements for this study. If carbon would have originated from the resin, this would likely be accompanied by additional problems such as polymers clogging up the sampling system. Such issues have not been observed during the measurements, and we thus assume no significant interferences due to the resin. Nitrate from the acid used represents a potential source of N, though since all solutions were diluted from a single batch of acid, this would also lead to a constant impact across all measurements. The peak-stripping procedure performed removes this potential CN interference, since these would introduce constant contributions to all mass 26 measurements.

All statistical analysis was performed using RStudio Version 1.1.453.

## RESULTS

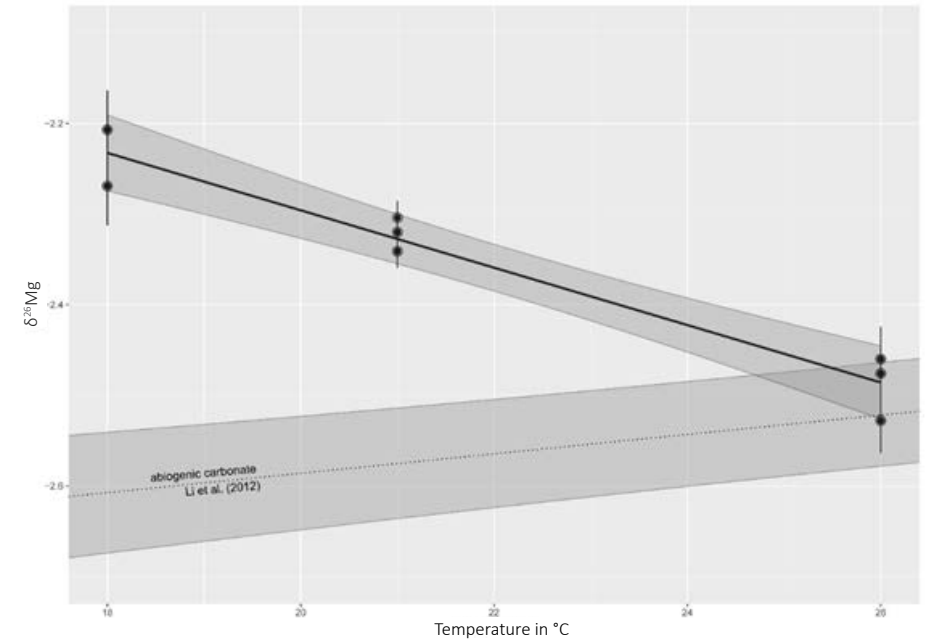
$\delta^{25}\text{Mg}$  and  $\delta^{26}\text{Mg}$  values and derived Mg/Ca from the temperature experiment

The culture medium was found to have  $\delta^{25}\text{Mg}$  and  $\delta^{26}\text{Mg}$  values of  $-0.44\text{‰}$  and  $-0.81\text{‰}$  (relative to DSM-3) respectively, which corresponds to open ocean seawater (Foster et al., 2010; Pogge von Strandmann, 2008; Ra and Kitagawa, 2007; Young and Galy, 2004). Values for  $\delta^{25}\text{Mg}$  and  $\delta^{26}\text{Mg}$  measured in the cultured foraminifera range from  $-1.29$  to  $-1.15\text{‰}$  and  $-2.48$  to  $-2.21\text{‰}$  respectively (Table 3). The  $\delta^{26}\text{Mg}$  values show a strong, negative correlation with temperature in *A. lessonii* ( $\delta^{26}\text{Mg} = -0.03 (\pm 0.003 \text{ se}) * T - 1.66 (\pm 0.07 \text{ se})$ ;  $p\text{-value} < 0.01$ , adjusted  $R^2 = 0.94$ ; Fig. 2).

**Table 3.** Mean  $\delta^{25}\text{Mg}$  and  $\delta^{26}\text{Mg}$  values ( $\pm 2$  standard deviations; SD) relative to DSM-3 measured in *Amphistegina lessonii* cultured at different temperatures.

Temperature (°C)	$\delta^{25}\text{Mg}_{\text{DSM-3}} \text{‰} (\pm 2\text{SD})$	$\delta^{26}\text{Mg}_{\text{DSM-3}} \text{‰} (\pm 2\text{SD})$
18	$-1.15 (\pm 0.03)$	$-2.20 (\pm 0.09)$
	$-1.17 (\pm 0.03)$	$-2.27 (\pm 0.09)$
21	$-1.22 (\pm 0.05)$	$-2.34 (\pm 0.04)$
	$-1.17 (\pm 0.05)$	$-2.30 (\pm 0.04)$
	$-1.20 (\pm 0.05)$	$-2.32 (\pm 0.04)$
26	$-1.27 (\pm 0.02)$	$-2.53 (\pm 0.07)$
	$-1.27 (\pm 0.02)$	$-2.48 (\pm 0.07)$
	$-1.29 (\pm 0.02)$	$-2.46 (\pm 0.07)$

The Mg/Ca values of the foraminifera was calculated using a species-specific temperature calibration (van Dijk et al., 2018), resulting in Mg/Ca ratios of 13.9, 19.0 and 27.4 mmol/mol for the specimens stemming from the 18, 21 and 26 °C treatment respectively.



**Figure 2.**  $\delta^{26}\text{Mg}$  values ( $\pm\text{SD}$ ) measured in foraminiferal calcite of cultured *Amphistegina lessonii* shows a strong, negative correlation with temperature. Since temperature was a controlled variable, error bars are within the width of the symbol. The grey area indicates the 95% confidence interval. The relationship observed can be described as  $\delta^{26}\text{Mg} = -0.03 * T - 1.66$  ( $p\text{-value} < 0.001$ , adjusted  $R^2 = 0.93$ ). This relationship is opposite to the one observed in some abiogenic carbonates (Li et al., 2012).

## Mg ISOTOPIC COMPOSITION OF FORAMINIFERA FROM THE INDO-PACIFIC AQUARIUM

The Mg-isotopic fractionation between species derived from the coral debris collected from the aquarium (kept at  $26.0 \pm 0.5$  °C during the last 20 years; Ernst et al., 2011) varies greatly between species, though the variability within the hyaline foraminifera appears to be larger than within the analysed species of porcelaneous foraminifera (Table 4). Mean  $\delta^{26}\text{Mg}_{\text{DSM-3}} \pm 2\text{SD}$  of the foraminiferal samples varies between  $-2.09 \pm 0.09$  ‰ and  $-2.88 \pm 0.03$  ‰ for the investigated hyaline species (*Amphistegina lessonii* and *Heterostegina depressa*) and ranges from  $-3.08 (\pm 0.07)$  to  $-3.00 (\pm 0.04)$  for the porcelaneous species (*Sorites orbitolis*, *Spiroculina* spp., *Quinqueloculina pseudoreticulina*, *Miliolinella labiosa*).

**Table 4.** Mean  $\delta^{25}\text{Mg}$  and  $\delta^{26}\text{Mg}$  values ( $\pm 2$  standard deviations; SD) relative to DSM-3 of a variety of benthic foraminiferal species collected from coral debris of the Indo-Pacific aquarium of Burgers' Zoo, The Netherlands.

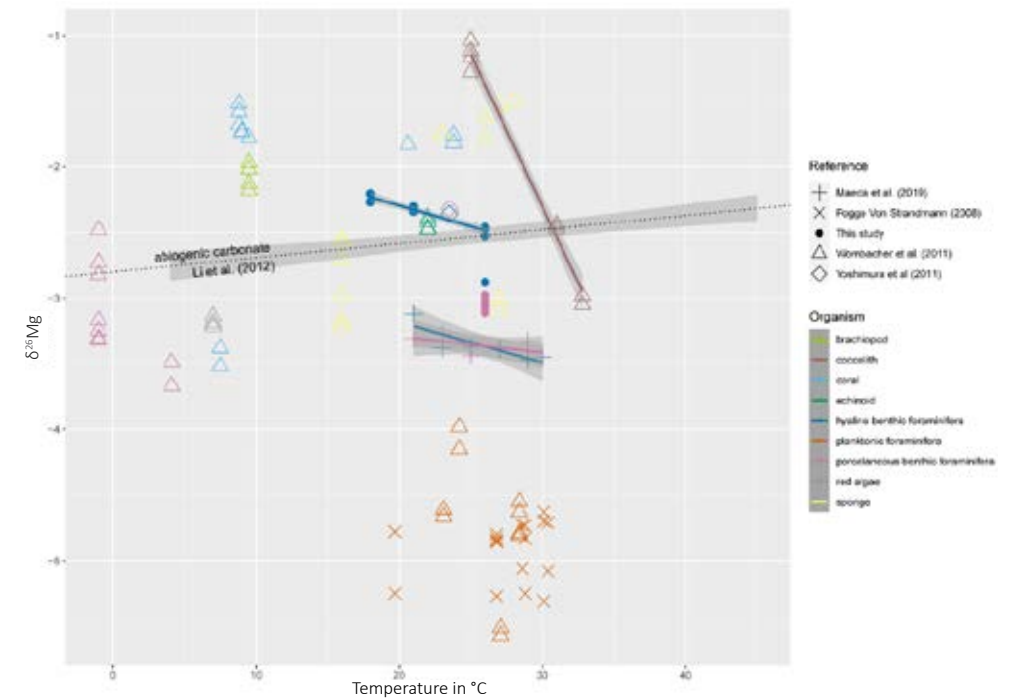
	Species	$\delta^{25}\text{Mg}_{\text{DSM-3}} \text{‰} (\pm 2\text{SD})$	$\delta^{26}\text{Mg}_{\text{DSM-3}} \text{‰} (\pm 2\text{SD})$
Hyaline species	<i>Amphistegina lessonii</i>	-1.08 ( $\pm 0.07$ )	-2.09 ( $\pm 0.09$ )
	<i>Heterostegina depressa</i>	-1.49 ( $\pm 0.03$ )	-2.88 ( $\pm 0.03$ )
Porcelaneous species	<i>Sorites orbitolis</i>	-1.56 ( $\pm 0.04$ )	-3.00 ( $\pm 0.04$ )
	<i>Spiroloculina</i> spp. ( <i>angluata</i> + <i>communis</i> )	-1.60 ( $\pm 0.03$ )	-3.08 ( $\pm 0.07$ )
	<i>Quinqueloculina pseudoreticulina</i>	-1.59 ( $\pm 0.03$ )	-3.06 ( $\pm 0.05$ )
	<i>Miliolinella labiosa</i>	-1.56 ( $\pm 0.04$ )	-3.06 ( $\pm 0.06$ )

## DISCUSSION

### $\delta^{26}\text{Mg}$ IN MARINE CALCIFIERS

A wide range in values in magnesium isotope ratios has been observed for marine calcifiers. Earlier studies on the relationship between temperature and  $\delta^{26}\text{Mg}$  values of biogenic and abiogenic calcium carbonate showed highly variable Mg isotope ratios and trends (Fig. 3). For sponges, a slight positive correlation with temperature is observed, though different species show different response to both absolute temperature as well as changes in sea water temperature. Previously published  $\delta^{26}\text{Mg}$  values for planktonic foraminifera range from -4 to -5.5‰ (Chang et al., 2004; Pogge von Strandmann, 2008; Wombacher et al., 2011), showing a slight negative relationship with temperature, which is not present when considering individual species. Although the isotopic composition of Mg in sea water is assumed to be rather uniform based on the residence time of Mg in seawater which is several orders of magnitude higher than ocean mixing time (Foster et al., 2010; Lécuyer, 2016), different biotic and abiotic processes result in highly variable isotope fractionation. Variability could be caused by kinetic effects or differences in precipitation rates (Immenhauser et al., 2010; Mavromatis et al., 2013) or by microscopic fluid inclusions that have been observed in inorganic calcite growth experiments (Saulnier et al., 2012). While fluid inclusions so far have not been observed in biogenic calcite, precipitation rates among marine calcifiers vary (e.g. Ullmann, 2016)

and are known to impact the isotopic composition of calcium carbonates (DePaolo, 2011). Well established estimates of carbonate precipitation rates would therefore be a valuable addition to take into account and could allow inter-taxa comparisons as well as deepen our understanding of Mg isotope incorporation, such data is unfortunately very difficult to obtain. The largest differences in fractionation are most likely due to biogenic processes though considering the large differences observed between organisms even under similar temperatures. Such a biogenic contribution to the overall Mg isotope fractionation is apparently variable amongst organisms and between species as  $\delta^{26}\text{Mg}$  can vary up to 4‰. For example,  $\delta^{26}\text{Mg}$  values for coccolithophores are reported as high as -1‰ (relative to DSM-3), whereas planktonic foraminifera have values as low as -5.5‰ (Chang et al., 2004; Pogge von Strandmann, 2008; Wombacher et al., 2011).

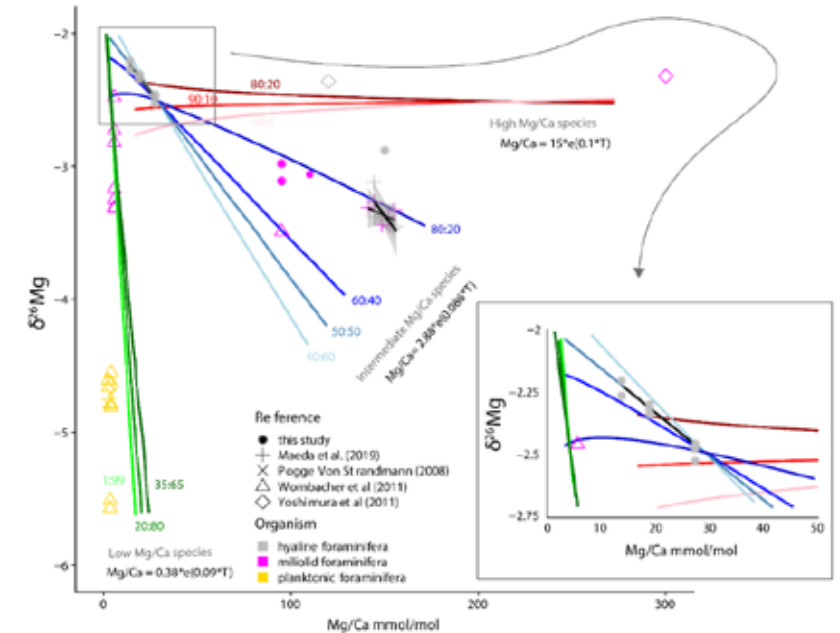


**Figure 3.**  $\delta^{26}\text{Mg}$  values versus temperature for different organisms from literature data (Maeda et al., 2019; Pogge von Strandmann, 2008; Wombacher et al., 2011; Yoshimura et al., 2011) and this study, showing different responses between groups of organisms. In addition to species specific relationships in benthic foraminifera (see Figure 1), a statistically significant relationship ( $p$ -values  $< 0.05$ ) can be observed for coccoliths ( $\delta^{26}\text{Mg} = -0.23 \cdot T + 4.66$ ,  $R^2 = 0.98$ ), and sponges ( $\delta^{26}\text{Mg} = 0.07 \cdot T - 4.0$ ,  $R^2 = 0.34$ ), while other groups such as corals show no clear trend and the  $\delta^{26}\text{Mg}$  values in abiogenic carbonates rise with temperature (Li et al., 2012). Error bars for *Amphistegina lessonii* are shown in Figure 2.

Abiogenic carbonate shows  $\delta^{26}\text{Mg}$  values between  $-2.8$  and  $-2.4$  ‰ with a slight positive correlation between temperature and both Mg incorporation and  $\delta^{26}\text{Mg}$  (Li et al., 2012): i.e., more Mg is incorporated at higher temperature, and the Mg is at the same time less depleted with respect to heavy isotopes. In contrast a strong negative relationship of  $\delta^{26}\text{Mg}$  with temperature has been observed for coccoliths (Wombacher et al., 2011). Since all coccolith samples were collected from the field, it was suggested by Wombacher et al. (2011) that the observed trend may, however, have been indirect. Here we observe a similar trend for intermediate Mg/Ca foraminifera from controlled growth experiments, suggesting that temperature somehow affects Mg isotopes in some marine calcifiers. Since biomineralization processes vary largely between taxa, conclusions drawn from foraminifera presented here cannot be transferred to other organisms without caution though.

#### $\delta^{26}\text{Mg}$ IN FORAMINIFERAL CALCITE AND THE EFFECT OF TEMPERATURE

Results from previous studies combined with ours show that foraminiferal  $\delta^{26}\text{Mg}$  values range between  $-5.5$  and  $-1$  ‰ (DSM-3; Chang et al., 2004; Maeda et al., 2019; Pogge von Strandmann, 2008; Wombacher et al., 2011; Yoshimura et al., 2011; Young and Galy, 2004). To investigate the relation between  $\delta^{26}\text{Mg}$  and Mg content, we plotted data from our study as well as previous studies, of which both these parameters were known or calculated through well-established calibrations, in Figure 4. On a species level, the  $\delta^{26}\text{Mg}$  values decrease with increasing Mg content, although the range in fractionation itself increases at lower Mg/Ca values. This suggests that for species with high Mg/Ca calcite shells, precipitation resembles more closely inorganic precipitation from an open sea water reservoir, whereas at low Mg/Ca values (i.e., when organisms discriminate against Mg-incorporation) fractionation can be either close to inorganic values (open system) or there may be strong offsets from sea water towards more negative values. This implies that the observed range at low Mg/Ca reflects mainly the biological control and to a lesser extent the inorganic fractionation effects, viz. for example the large range in  $\delta^{26}\text{Mg}$  for planktonic foraminifera at relatively uniform Mg/Ca values (Fig. 4; Wombacher et al., 2011; Yoshimura et al., 2011).



**Figure 4.** Modelled Mg/Ca versus  $\delta^{26}\text{Mg}$  calculated over a range of temperatures using the species-specific temperature calibrations for a number of (hypothetical) species (red, blue and green lines). Lines indicate isotopic signatures as a consequence of different relative amounts of low versus high Mg/Ca bands (high:low). Fitted calibration lines (black), including uncertainty intervals are added for *A. lessonii* (this study) as well as *A. kudakajimensis* and *C. gaudichaudii* (Maeda et al., 2019). Also included is published data (Maeda et al., 2019; Pogge von Strandmann, 2008; Wombacher et al., 2011; Yoshimura et al., 2011) for comparison with the model output. When information about Mg/Ca was not available from the same study as the  $\delta^{26}\text{Mg}$  values, Mg/Ca was estimated using accompanying temperature information and published calibrations (Anand et al., 2003; de Nooijer et al., 2017b; Nürnberg et al., 1996; Raja et al., 2007; Sadekov et al., 2014). Mg/Ca of specimens from the controlled culture experiment are based on the Mg/Ca-temperature calibration of van Dijk et al. (2018).

We show a distinct negative relation (slope  $-0.03$  ‰/°C) between foraminiferal  $\delta^{26}\text{Mg}$  and Mg/Ca for the hyaline species *A. lessonii* (Fig. 2). The overall trend is about three times more sensitive as observed for porcelaneous *Amphisorus kudakajimensis* (slope  $-0.01$  ‰/°C; Maeda et al., 2019). Such a difference most likely reflects a difference in the biomineralization between these species, which is also evident in the contrasting Mg/Ca of these species: *A. kudakajimensis* typically has Mg/Ca ratios of  $\sim 150$  mmol/mol, whereas *A. lessonii* has an order of magnitude lower Mg/Ca ratio, typically of 20-30 mmol/mol (e.g. van Dijk et al., 2019; Geerken et al., 2019). The sensitivity of the isotopic composition of incorporated Mg to temperature also varies between these species: *A. lessonii* being less sensitive (slopes of the calibration regression being  $1.69$  mmol/mol/°C



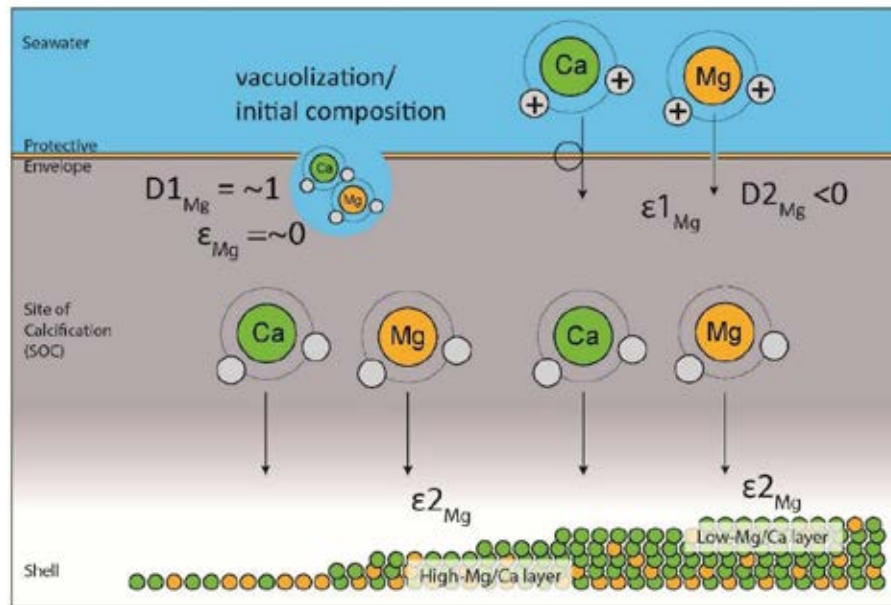
in *A. lessonii* (van Dijk et al., 2018) compared to 2.74 mmol/mol/°C in *A. kudakajimensis* (Maeda et al., 2017). Overall, the isotopic sensitivity seems to increase with organismal fractionation against Mg during biomineralization.

The higher temperature sensitivity of both Mg/Ca and Mg isotopes in *A. lessonii* suggests that the incorporation and fractionation of Mg is intrinsically coupled, which is supported by the observation that most isotopically depleted values are associated with species most depleted in Mg (Fig. 4). The process-based explanation for the observed relation between Mg incorporated and the isotopic fractionation will also have to relate to the banded within-wall Mg distribution, which occurs in alternating low- and high-concentration bands in many benthic and planktonic species (Bentov and Erez, 2006; Erez, 2003; Fehrenbacher et al., 2017; Sadekov et al., 2005; Steinhardt et al., 2015). Although the exact mechanism responsible for Mg-transport during calcification remains to be fully determined, it is generally thought that both precipitation from seawater and from a Mg-depleted fluid are involved. In many reports, the higher Element/Ca ratio (El/Ca) of elements such as Mg, Na or K are found in the first layers precipitated and the lower El/Ca bands are precipitated later (e.g. van Dijk et al., 2019a; Geerken et al., 2019). There are some exceptions with reports on highest El/Ca at the end of chamber wall formation (Jonkers et al., 2016) or alternations of high- and low-El/Ca bands in the spherical chamber of *Orbulina universa* (Spero et al., 2015). Here we use these observations and recent concepts in foraminiferal biomineralization models to explore the coupled Mg/Ca and Mg isotope behaviour with a simple two-endmember-mixing model resembling foraminiferal chamber formation.

## FORAMINIFERAL MG INCORPORATION DURING BIOMINERALIZATION

Calcifying perforate foraminifera form an organic, protective envelope around their shell that shields the site of calcification from the direct environment and allows for chemical manipulation, such as increasing the pH of the calcifying fluid (Erez, 2003; Jørgensen et al., 1985; de Nooijer et al., 2009). The Mg isotope signal of the first calcium carbonate formed during biomineralization likely represents that of inorganic calcium carbonate formed from the sea water trapped by the protective envelope (Fig. 5). As the newly formed carbonate layer grows, more Ca<sup>2+</sup> ions are transported from the sea water to the site of calcification through selective calcium ion channels in the protective envelope. While these channels mainly transport calcium ions, they likely bring a small amount of Mg<sup>2+</sup> ions into the site of calcification. While these transmembrane transport channels have not yet been identified in foraminifera, they have long been suspected (Marchitto et al., 2018; Nehrke et al., 2013) and Ca-ATPases with a strong Ca-selectivity

over Mg<sup>2+</sup> are known from other organisms (Drake et al., 1996; Xiang et al., 2007). It is also likely that as this transport happens, these channels also discriminate against heavier Mg isotopes, which leads to an increasingly depleted δ<sup>26</sup>Mg signal being recorded in the calcium carbonate. Channel activity and/or selectivity could vary as a function of temperature. Bulk shell δ<sup>26</sup>Mg represents a mixed signal, consisting of the initial layer of carbonate, formed from the sea water Mg ions at the start of calcification, and the later leaked-in Mg ions transported through the discriminating ion channels. The bulk shell δ<sup>26</sup>Mg therefore depends on the isotopic composition of the sea water (determining the first endmember), the fractionation caused by the ion channels (determining the second endmember) as well as the ratio of initial to derived carbonate formed during calcification, which determines mixing of the endmembers. Both endmembers differ in the process that supplies the Mg<sup>2+</sup> to the calcifying fluid, but once the Mg has arrived in the calcifying fluid, precipitation of both endmembers will be impacted by the same processes that also affect inorganic calcite precipitation such as Rayleigh fractionation. As modelled results show, the effect of Rayleigh fractionation on the bulk shell δ<sup>26</sup>Mg signal would cause a slightly, but significantly positive correlation with temperature (Pogge von Strandmann, 2008), which is the opposite of the trend observed here. It can therefore be concluded that the biogenic effect caused by the transmembrane transport is large enough to overcome possible effects caused by Rayleigh fractionation and still be measurable. Rayleigh fractionation therefore only plays a minor role in foraminiferal δ<sup>26</sup>Mg signals, which appear to be largely controlled by transmembrane transport, similarly to foraminiferal Mg/Ca.



**Figure 5.** Summary of Mg fractionation during hyaline foraminiferal calcification.

In our model, for simplicity, we assume that species primarily differ in their average Mg/Ca as a consequence of the ratio of seawater- and ion-pump derived Mg. Species with calcite Mg/Ca values of >150 mmol/mol likely precipitate from a solution with a seawater-like Mg concentration (e.g., miliolids or high-Mg/Ca larger benthic foraminifers). Species with lowest Mg/Ca values (i.e., ~1-5 mmol/mol), make most of their carbonate with ion-pump derived Ca and hence also (depleted) Mg. Using the here measured effect of temperature on the Mg/Ca- $\delta^{26}\text{Mg}$  relationship for *A. lessonii*, and assuming a 50/50 ratio of Mg in low- and high-Mg carbonate bands (Geerken et al., 2019) allows deconvolving the isotope values of both endmembers as function of temperature. Using EPMA analyses, Geerken et al. (2019) established that roughly 50% of the Mg is part of the high Mg-carbonate and the remaining 50% is part of the low Mg carbonate in *A. lessonii*. Moreover, average Mg/Ca for *A. lessonii* can be described as (van Dijk et al., 2018; Geerken et al., 2019):

$$\text{Mg/Ca} = 2.88 * e^{(0.086*T)}$$

Where Mg/Ca is in mmol/mol and  $T$  is the temperature in °C. The Mg/Ca of the high and

low phases can be calculated:

$$\text{Mg/Ca}_{\text{high band}} = \frac{\text{Mg/Ca}_{\text{total}}}{p + \frac{1-p}{v}}$$

Where  $p$  is the proportion of Mg in the high-Mg/Ca band, which likely varies from species to species but could also vary between specimens of the same species, and  $v$  refers to the ratio between Mg/Ca values in the high- and in the low-Mg/Ca band (2.8; Geerken et al., 2019), but does not affect the relative contribution of both layers on the isotopes. From our results (Fig. 2), the effect of temperature on the  $\delta^{26}\text{Mg}$  in *A. lessonii* can be described by the following equation with slope and intercept given with  $\pm$  standard error:

$$\delta^{26}\text{Mg} = -0.032 (\pm 0.003) * T - 1.66 (\pm 0.073)$$

With the  $\delta^{26}\text{Mg}$ /temperature-dependency of inorganically precipitated calcite (Li et al., 2012), the  $\delta^{26}\text{Mg}$  of the high-Mg/Ca band can hence be approximated:

$$\delta^{26}\text{Mg}_{\text{high band}} = 0.0107 (\pm 0.0018) * T - 2.80 (\pm 0.05)$$

And the  $\delta^{26}\text{Mg}_{\text{low band}}$  is simply:

$$\delta^{26}\text{Mg}_{\text{low band}} = \frac{\text{Mg/Ca} - \delta^{26}\text{Mg}_{\text{high band}} * p}{1 - p}$$

The  $\delta^{26}\text{Mg}$  of the calculated low-Mg/Ca band then can be used to calculate the possible Mg/Ca-  $\delta^{26}\text{Mg}$  for other species with a different Mg/Ca- $T$  calibration and/ or species with a considerably lower or higher Mg/Ca. First, applying different relative contributions of the low and high Mg/Ca bands clearly show that this mixing model explains the values observed for *A. lessonii*, and also can be extended to *A. kudakajimensis* appeared and *C. gaudichaudii* using a relative contribution of 80% of high Mg carbonate. This higher

relative contribution is in line with the higher overall Mg/Ca values for these species.

Fig. 4 shows a number of possible, modelled Mg/Ca- $\delta^{26}\text{Mg}$  relationships for a range of temperatures for species with fundamentally different Mg-incorporation. The other Mg/Ca-T calibrations are here assumed to resemble those of planktonic species (i.e.,  $\text{Mg/Ca} = 0.38 \cdot e^{(0.09 \cdot T)}$ , Anand et al., 2003) and those of high-Mg/Ca species (i.e.,  $\text{Mg/Ca} = 15 \cdot e^{(0.1 \cdot T)}$ , cf. Wit et al., 2012). This essentially renders the Mg/Ca- $\delta^{26}\text{Mg}$  relationship a function of  $p$  (i.e., the proportion of Mg in the high-Mg/Ca band).

The two end-member model presented here, which incorporates the earlier established inorganic fractionation and the biological process which either pumps in Ca and/or removes Mg from the SOC, can not only explain the temperature dependence we observed for *A. lessonii*, but also the differences between the different species. When all these organisms or foraminifera would use the same ion pump mechanism one would expect a single correlation between Mg/Ca and  $\delta^{26}\text{Mg}$ . This is clearly not what is observed here. Although the fact that at high Mg/Ca values  $\delta^{26}\text{Mg}$  consistently plots close to the inorganic values, whereas at low Mg/Ca values a large range in  $\delta^{26}\text{Mg}$  is observed implies a major biological imprint, using two different phases only suffices to explain all data measured until now for foraminifera. Clearly this does not imply that these two processes are the only ones involved in Mg incorporation and hence fractionation. The offsets between species in our model with a variable fractionation during ion transport for the low Mg/Ca phase does highlight the importance of ion transport during foraminiferal calcification. From a proxy-perspective, the  $\delta^{26}\text{Mg}$  adds to the mechanistic understanding of the relation between Mg/Ca, temperature and biomineralization.

## CONCLUSION

Here we tested the effect of temperature on  $\delta^{26}\text{Mg}$  in the large benthic, symbiont bearing foraminifera *Amphistegina lessonii*. Results show a significant negative relationship with higher temperatures leading to a more negative isotopic signal in the foraminiferal calcite. This phenomenon can be explained by a combination of two pathways that lead to the incorporation of Mg into the carbonate. Initially formed carbonate comprises  $\text{Mg}^{2+}$  ions stemming from the sea water trapped by the protective envelope surrounding the foraminifera during calcification. This isotopic signal gets modified by new  $\text{Mg}^{2+}$  ions reaching the site of calcification through  $\text{Ca}^{2+}$  ion channels that discriminate against heavier isotopes and thus cause fractionation. The former process explains the Mg isotopes in the high-Mg band commonly found in Rotaliid foraminifera and follows the inorganic  $\delta^{26}\text{Mg}$ -temperature relationship. The second process adds a

less depleted phase to the shell wall. Inter-species differences in both average  $\delta^{26}\text{Mg}$  and Mg/Ca can thus be explained by differences in relative contribution of these two phases.

## AUTHOR CONTRIBUTIONS

The study was designed by LKD, IvD, LdN and GJR. The temperature experiment was prepared and performed by LKD and BZ, the geochemical analyses at NIOZ were done by BvdW and BZ. Geochemical analyses at MARUM were performed by FKW, the species identification and sample preparation were done by IvD. The model was developed by GJR with input from LdN and LKD, the manuscript was drafted by LKD with contributions from all authors.

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# CHAPTER FIVE

## EVALUATION OF OXYGEN ISOTOPES AND TRACE ELEMENTS IN PLANKTONIC FORA- MINIFERA FROM THE MEDITERRANEAN SEA AS RECORDERS OF SEAWATER OXYGEN ISOTOPES AND SALINITY

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Evaluation of oxygen isotopes and trace elements in planktonic foraminifera  
from the Mediterranean Sea as recorders of seawater oxygen isotopes and salinity.  
*Climate of the Past*, 16(6), 2401–2414. <https://doi.org/10.5194/cp-16-2401-2020>

### ABSTRACT

The Mediterranean Sea is characterized by a relatively strong west to east salinity gradient, which makes it an area suitable to test the effect of salinity on foraminiferal shell geochemistry. We collected living specimens of the planktonic foraminifer *Globigerinoides ruber albus* to analyse the relation between element/Ca ratios, stable oxygen isotopes of their shells and surface seawater salinity, isotopic composition, and temperature. The oxygen isotopes of sea surface water correlate with salinity in the Mediterranean also during winter, when sampled for this study. Sea water oxygen and hydrogen isotopes are positively correlated in both the eastern and western Mediterranean Sea, though especially in the eastern part the relationship differs from values reported previously for that area. The slope between salinity and seawater oxygen isotopes is lower than previously published. Still, despite the rather modest slope, seawater and foraminiferal carbonate oxygen isotopes are correlated in our dataset although with large residuals and high residual variability. This scatter can be due to either biological variability in vital effects or environmental variability. Numerical models backtracking particles show ocean current driven mixing of particles of different origin might



dampen sensitivity and could result in an offset caused by horizontal transport. Results show that Na/Ca is positively correlated to salinity and independent of temperature. Foraminiferal Mg/Ca increases with temperature, as expected, and in line with earlier calibrations, also in the high salinity environment. By using living foraminifera during winter, the previously established Mg/Ca-temperature calibration is extended to temperatures below 18 °C, which is a fundamental prerequisite of using single foraminifera for reconstructing past seasonality.

## INTRODUCTION

Ocean circulation plays an important role in Earth's climate, by redistributing heat and also by impacting global biogeochemical cycles. Seawater temperature and salinity are key parameters for reconstructing ocean circulation, since together they determine seawater density and thereby large-scale circulation patterns, including a substantial part of meridional overturning circulation. Reconstruction of past ocean environments largely relies on so-called proxy calibrations in which a variable which can be measured in the geological record is related to a target environmental parameter. The incorporation of trace metals in foraminiferal shell carbonate, for example, is a popular tool to reconstruct past ocean parameters. More specifically, the incorporation of Mg (often expressed as the calcite's Mg/Ca) increases exponentially with seawater temperature, as first observed in culture studies (Nürnberg et al., 1996) and later confirmed by field calibrations (Anand et al., 2003).

In addition to temperature, salinity and inorganic carbon chemistry also affect Mg/Ca in some species of foraminifera (Allison et al., 2011; Dueñas-Bohórquez et al., 2011; Geerken et al., 2018; Gray et al., 2018; Hönisch et al., 2013; Kiskirek et al., 2008; Lea et al., 1999). For the best possible accuracy such effects need to be corrected for when using foraminiferal Mg/Ca for the reconstruction of temperature, which calls for independent proxies for these other environmental parameters.

Currently, salinity is often reconstructed through indirect relationships with other variables, such as the ratio of stable oxygen isotopes of sea water, which are recorded in planktonic foraminifera (Rohling, 2007), although direct approaches have also been suggested recently (Bertlich et al., 2018; Wit et al., 2013). Since seawater oxygen isotope ratio and salinity are both affected by evaporation and precipitation, the two often are linearly related (Bahr et al., 2013; Rohling, 2007), with their calibration depending on local conditions. If foraminifera precipitate their calcite in equilibrium with

respect to sea water oxygen isotopes, their  $\delta^{18}\text{O}$  should reflect that of the seawater, and hence salinity. However, as seawater temperature affects stable oxygen isotope fractionation during calcification (McCrea, 1950; Urey et al., 1951) independent temperature reconstructions are needed to estimate seawater  $\delta^{18}\text{O}$  from  $\delta^{18}\text{O}_{\text{calcite}}$  (Rohling, 2007). Independent temperature reconstructions can be based for example on organic proxies such as  $\text{U}^{K}_{37}$  (Pahl and Wakeham, 1987), TEX86 (Schouten et al., 2006) or the Mg/Ca of the foraminifera themselves (Elderfield and Ganssen, 2000; Mashiotta et al., 1999). Accuracy and precision of such reconstructions is debated because propagation of errors from combined inaccuracies of the analyses and the uncertainties in calibrations due to combining several proxies, is difficult to assess and seems too large for meaningful reconstructions of changes in salinity over time (Rohling, 2007). Because of the lack of a suitable alternative approach, the use of Mg/Ca to determine the temperature effect of foraminiferal  $\delta^{18}\text{O}$  continues to be applied in settings which are prone to large changes in salinity such as the Mediterranean Sea. This calls for an independent in-situ calibration in which all the involved parameters are measured and not determined by proxy-relationships.

Culture experiments using the benthic, symbiont-barren *Ammonia tepida* (Wit et al., 2013) and the planktonic *Globigerinoides ruber* (pink) (Allen et al., 2016), showed that Na incorporation in foraminiferal shell carbonate is positively correlated with sea water salinity. A field calibration confirmed this positive correlation for the planktonic foraminiferal species *G. ruber albus* (Mezger et al., 2016), as well as for *Trilobatus sacculifer* (previously called *Globigerinoides sacculifer*) in the Red Sea and the Atlantic Ocean (Bertlich et al., 2018; Mezger et al., 2016). Comparison of Na/Ca-salinity calibrations shows, however, that absolute Na/Ca values and also sensitivities to salinity vary between species (Mezger et al., 2016).

When using field calibrations to constrain accuracy and precision of potential reconstruction approaches, it is important to also consider the potential impact of lateral transport of foraminifera due to (ocean) currents. Foraminifera collected at a specific sampling location might actually have added the majority of their shell carbonate at a different location and hence under different environmental conditions as they have been transported to the sampling location. This may add to the uncertainty in the variable to cross-correlate against or even introduce a bias in the resulting calibration. Recently this has been suggested for dinoflagellate cysts (Nooteboom et al., 2019) and planktonic foraminifera, collected from the water column (Ganssen and Kroon, 1991), from sediment (van Sebille et al., 2015) and also from sediment traps (Steinhardt et al.,

2014), but can also be applied to specimens collected living from the sea surface.

Here we used a plankton pump and sea water samples collected from the Mediterranean Sea in January and February of 2016 to test viability of deconvolving salinity from combined temperature and sea water oxygen isotope reconstructions. We also investigate the potential of the newly developed salinity proxy Na/Ca in the Mediterranean Sea. Using samples collected in winter we also extend the calibration of Mg/Ca to sea water temperature for *G. ruber albus* towards its lower temperature tolerance limits (14°C; Bijma et al. (1990)), which is essential for the application of this species for past seasonality reconstructions.

## MATERIALS AND METHODS

During two cruises (NESSC Cruises 64PE406 and 64PE407, RV Pelagia) between January 12th and February 25th in 2016, a total of 98 plankton samples were collected from the surface waters of the Mediterranean Sea along an east-west transect using a plankton pump system (Ottens, 1992). Surface water was continuously pumped on board from 5m water depth and lead through a plankton net with 100 µm mesh size. Replacing the cod-end every 6h (filtering 57m<sup>3</sup> of sea water on average, constantly monitored using a water gauge), accumulated samples were washed out of the net into a 90 µm sieve, rinsed thoroughly with deionized water to remove smaller particles as well as salts, and subsequently stored onboard at -80°C. At NIOZ all plankton samples were then freeze-dried, and dry oxidation by low temperature ashing (100°C) was used to combust the organic material while minimizing potential impacts on carbonate trace metal concentrations and δ<sup>18</sup>O (Fallet et al., 2009). After ashing, samples were rinsed again thoroughly with de-ionized water and ethanol to remove potential ash residues. A variety of samples containing specimens of *G. ruber albus* (Morard et al., 2019) was selected to cover a large range in salinities and temperatures. Specimens used for analyses were selected from the size fraction 150 - 250 µm, even though it has been reported that at this size fraction *G. ruber albus* and *Globigerinoides elongatus* cannot always be confidently distinguished due to similar morphology (Aurahs et al., 2011). Surface seawater samples for stable oxygen isotopes were collected every 60 minutes from the same pump, resulting in a set of 309 samples. A volume of 2 ml was stored without headspace at 4°C during the cruise to be analyzed at the home laboratory.

The elemental ratio of the final foraminiferal chamber (named the F-chamber) of individual shells were measured by laser ablation quadrupole inductively coupled plasma mass spectrometry (LA-Q-ICP-MS) using a circular spot with a diameter of 60-

80 µm, depending on the size of the last chamber. The laser system (NWR193UC, New Wave Research) at Royal NIOZ was used in combination with a two-volume sample cell (TV2), which allows detecting variability in elemental ratios within the foraminiferal chamber wall due to a short wash-out time of 1.8s (van Dijk et al., 2017b). Ablating only F-chambers minimizes sampling of older carbonate that might have formed under different environmental conditions due to lateral and vertical transport. All specimens were ablated with an energy density of 1±0.1 J/cm<sup>2</sup> and a repetition rate of 6Hz in a helium environment. A 0.7L/m helium flow transported the resulting aerosol to an in-house-built smoothing device before entering the quadrupole ICP-MS (iCAP-Q, Thermo Fisher Scientific). Masses 7Li, 11B, 23Na, 24Mg, 25Mg, 27Al, 43Ca, 44Ca, 57Fe, 88Sr, 137Ba and 238U were monitored, 44Ca served as an internal standard for quantification of the associated elements. The synthetic carbonate standard MACS-3 was used for calibration, in addition carbonate standards JcP-1, JcT-1, NFHS1 (NIOZ foraminifera house standard; Mezger et al., 2016) as well as glass standards SRM NIST610 and NIST612 were used for monitoring data quality. Accuracy of the analyses was 97%, while precision was 3.0% for Mg and 2.4% for Na measurements.

Stable oxygen and carbon isotopes of foraminiferal calcite were measured on groups of whole specimens different from those used for LA-Q-ICP-MS, using an automated carbonate device (Thermo Kiel IV) which was connected to Thermo Finnigan MAT 253 Dual Inlet Isotope Ratio Mass Spectrometer (IRMS). The NBS 19 limestone was used as a calibration standard, the NFHS1 standard was used for drift detection and correction. The standard deviation and offset of the NBS19 and the NFHS-1 were always within 0.1‰ for δ<sup>18</sup>O.

Due to the large amount of material required (20 to 40 µg) and the small amount of specimens present in the samples, specimens from different samples sometimes needed to be combined. This resulted, for example, in combining 12 and 8 µg of foraminifera from two adjacent transects and hence, the average temperature, salinity and δ<sup>18</sup>O seawater for these transects was calculated based on the relative contribution of the foraminiferal weight of the individual transects (i.e., 60 and 40 % respectively). Sea water oxygen and hydrogen stable isotopes were analysed with the Liquid Water Isotope Analyser (LWIA; Los Gatos Research Model 912-0008). This system measures the water samples using Off-Axis Integrated-Cavity Output Spectroscopy (OA-ICOS). The LWIA was connected with a GC PAL from CTC Analytics to inject 1 µl per measurement. To achieve this, the GCPAL was equipped with a 1.2 µl Hamilton syringe. In-House standards (S35, S45, NSW, LGR5 and double distilled water) were calibrated against VSMOW2-, VSLAP2- and GISP2- standard water obtained from IAEA in Vienna, using

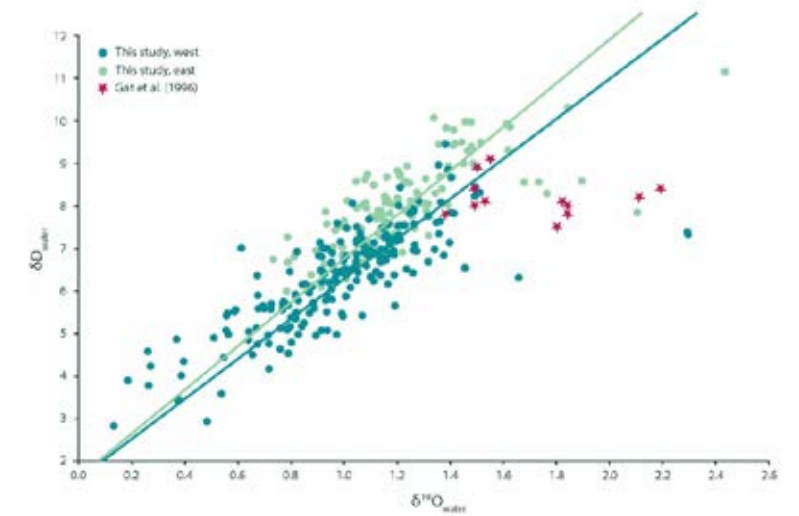
the same setup. The use of standard water VSMOW2, which has  $\delta^{18}\text{O}$  values identical to the older SMOW standard, allows for simple comparison with older data that was calibrated using SMOW, without additional corrections. Every sample and standard were measured 14 times sequentially, the first four runs were only used to flush the system while the last 10 measurements were used for the analysis. Additionally, between every sample or standard, the sample introduction line was rinsed with double distilled water. Data were processed with LGR LWIA Post Processor Software v. 3.0.0.88. Average standard deviation per sample was 0.14‰ for oxygen isotope measurements and 0.71‰ for hydrogen isotope measurements.

The likely provenance of the foraminifera sampled was computed by backtracking virtual particles in a high-resolution ocean model. For this, we used the Copernicus Marine Environmental Monitoring Service (CMEMS) Global Reanalysis model. The ocean surface currents, temperature and salinity are available at daily resolution and 1/12-degree horizontal resolution. In these fields, we backtracked particles using the OceanParcels v2.1.1 software (Delandmeter and van Sebille, 2019; Lange and van Sebille, 2017). We released 10,000 particles equally spaced between the start and end locations of 25 of the transects (i.e., all for which there were sufficient foraminiferal specimens for isotope analysis), on the day these transects were sampled, and tracked the particles back for 30 days with a 4th order Runge-Kutta algorithm with a 1-hour time step, storing local temperature, salinity, and location for each particle every day. To avoid beaching of particles, we used an unbeaching Kernel similar to that in Delandmeter and van Sebille (2019). The full code of the simulations is available at [https://github.com/OceanParcels/MedForams\\_Daemmer/](https://github.com/OceanParcels/MedForams_Daemmer/).

## RESULTS

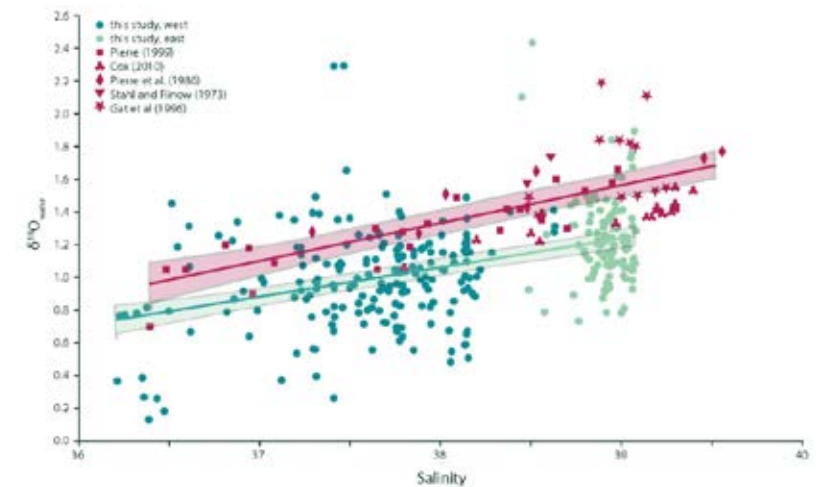
### MEDITERRANEAN SEA GEOCHEMISTRY

The sampled East-West transect spans a salinity gradient from 39.2 to 36.2 and an accompanying temperature gradient from 19°C (east) to 14°C. The 6 hour-intervals represented on average a distance of 57 kilometres (min 0 km, max 117 km). On average, this resulted in an internal variability of 0.14 salinity units and 0.33°C for each of the 98 transects.



**Figure 1.** The  $\delta\text{D}$  of the Mediterranean surface seawater is positively correlated with the local  $\delta^{18}\text{O}$ . The orthogonal regression of the western Mediterranean can be described as

$\delta\text{D}_{\text{water}} = 4.72 * \delta^{18}\text{O}_{\text{water}} + 1.67$  (dark green). The eastern Mediterranean is very similar to the western basin, and the relationship between seawater  $\delta^{18}\text{O}$  and  $\delta\text{D}$  is  $\delta\text{D}_{\text{water}} = 5.19 * \delta^{18}\text{O}_{\text{water}} + 1.68$  (light green) here. Statistically they cannot be told apart. This was determined using a bootstrapping approach that generated 100 slopes and intercepts for both the eastern and the western dataset and subsequent *t* testing using the mean and standard deviation of both groups of slopes and intercepts, which resulted in *p* values  $> 0.05$ . In both areas, the relationship is different from the observations made by Gat et al. (1996), whose dataset suggested no statistically significant relationship between  $\delta\text{D}$  and  $\delta^{18}\text{O}$  of the seawater (*p* value  $> 0.05$ ).



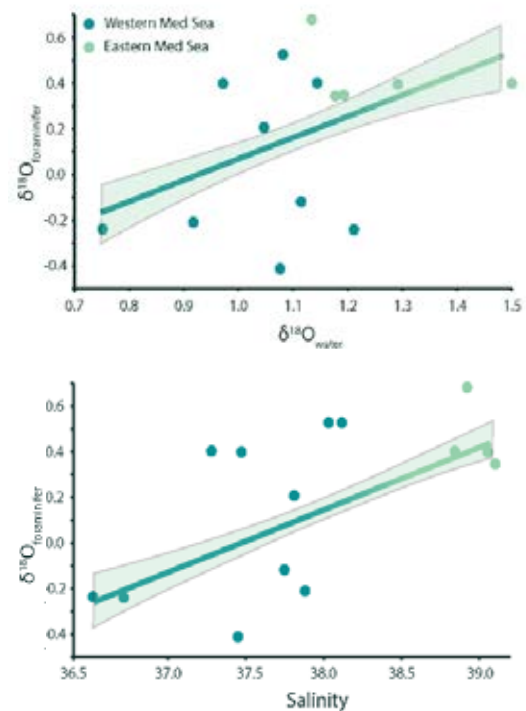
**Figure 2.** Surface seawater  $\delta^{18}\text{O}$  is positively correlated with sea surface salinity in the Mediterranean Sea, and the relationship observed can be described as linear regression  $\delta^{18}\text{O}_{\text{water}} =$

$0.17 * S - 5.39$  (*p* value  $< 0.001$ , adjusted  $R^2=0.17$ ). Previously published data can be combined into one dataset with a similar relationship with a slightly steeper slope, which is offset towards relatively higher  $\delta^{18}\text{O}$  ( $\delta^{18}\text{O}_{\text{water}} = 0.22 * S - 7.19$ ; *p* value  $< 0.001$ , adjusted  $R^2=0.48$ ). The two regression lines are significantly different from each other (ANOVA *p* value  $< 0.01$ ).

Measured sea water  $\delta D$  values show a range from 2.83 to 9.46‰ VSMOW in the western Mediterranean Sea and from 5.98 to 11.15‰ VSMOW in the east. Values from the individual transects were used in combination with the  $\delta^{18}O_{\text{water}}$  to check for internal consistency (Fig. 1). The  $\delta^{18}O$  values of the seawater varies between 0.13 and 2.29‰ VSMOW in the west, and between 0.73 and 2.43‰ VSMOW in the east (Fig. 1). In our dataset,  $\delta^{18}O$  and  $\delta D$  of the water are positively correlated in both the western and eastern part of the Mediterranean Sea (Fig. 1). The sensitivities of the  $\delta D$  to  $\delta^{18}O$  correlations are, indistinguishable. The sea water oxygen isotopes are also linearly correlated with sea water salinity (Fig. 2) and do not show an offset between the eastern and western basins ( $p$ -value < 0.001;  $R^2 = 0.17$ ).

### FORAMINIFERAL GEOCHEMISTRY

The foraminiferal oxygen isotope ratios ( $\delta^{18}O_{\text{foraminifer}}$ ) range from -0.41 to 0.68‰ and are significantly correlated to seawater oxygen isotope ratio (Fig. 3 a), albeit with much scatter ( $R^2 = 0.42$ ,  $p$ -value < 0.001). The  $\delta^{18}O_{\text{foraminifer}}$  are also positively correlated with sea surface salinity (Fig. 3 b) showing a similarly large amount of scatter ( $R^2 = 0.44$ ,  $p$ -value < 0.001).

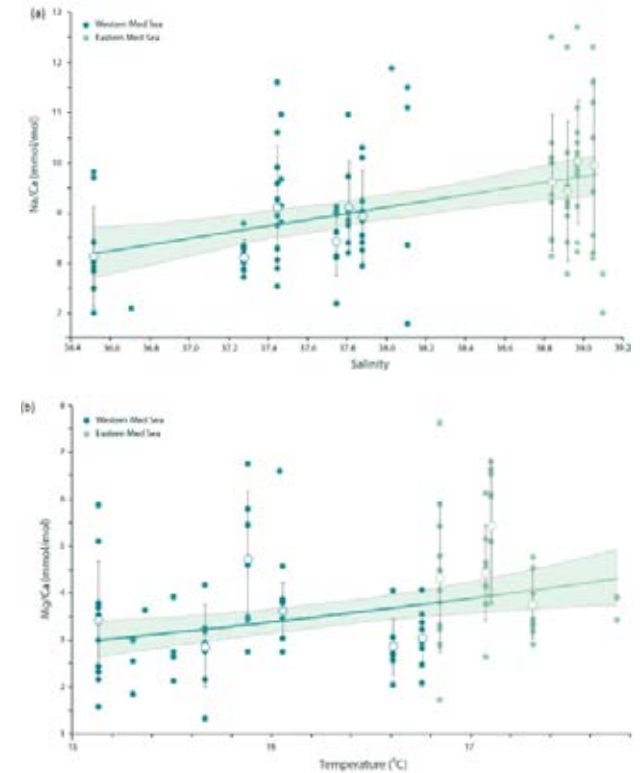


**Figure 3.** *G. ruber albus*  $\delta^{18}O$  measurements are positively correlated ( $p$ -value < 0.001) to both sea water  $\delta^{18}O$  (a) and salinity (b). The relationships can be described using the following equations:  $\delta^{18}O_{\text{foraminifera}} = 0.28 * S - 10.59$  (adjusted  $R^2 = 0.42$ ) and  $\delta^{18}O_{\text{foraminifera}} = 0.95 * \delta^{18}O_{\text{water}} - 0.89$  (adjusted  $R^2 = 0.24$ ). The relationship between  $\delta^{18}O_{\text{water}}$  and salinity in this subset of samples is linear and comparable to that of the entire dataset ( $\delta^{18}O_{\text{water}} = 0.13 * S - 3.91$ ;  $p$ -value < 0.05,  $R^2 = 0.37$ ).

### NA/CA VS SALINITY

Na/Ca values measured on individual F-chambers of *G. ruber albus* from the Mediterranean Sea range from 6.8 to 12.7 mmol/mol and are positively correlated with sea surface salinity (Fig. 4a). The variability between individuals (1-2 mmol/mol) observed within transects is orders of magnitude higher than the analytical uncertainty (RSD of 5%) and is also higher than the uncertainty in the slope of the Na/Ca-salinity calibration (Fig. 4a).

**Figure 4.** (a) Na/Ca measured in *G. ruber albus* F-chambers collected as living specimens from the eastern and western Mediterranean Sea correlates well with local salinity ( $p$ -value < 0.001,  $Na/Ca = 0.60 * S - 13.84$ ), even though a large natural spread of elemental composition around the mean values per station exists ( $R^2 = 0.13$ ). For salinities with more than 5 individual Na/Ca measurements, hollow circles with whiskers indicate average values and standard deviations. (b) Mg/Ca in F-chambers of *G. ruber albus* specimens collected from the water column of the Mediterranean Sea is positively correlated with sea surface temperature and can be described with the exponential relationship  $Mg/Ca = 0.37 * \exp(0.14 * T)$ . For temperatures with more than 5 individual Mg/Ca measurements, hollow circles with whiskers indicate average values and standard deviations. Regression lines were calculated using all individual data points.



### MG/CA VS TEMPERATURE

Mg/Ca-values measured on individual F-chambers of *G. ruber albus* from the Mediterranean Sea range from 1.34 to 7.63 mmol/mol and are positively correlated with in-situ measured sea surface temperatures, although the temperature range sampled during wintertime was rather narrow (Fig. 4b).



## PARTICLE BACKTRACKING

Particle backtracking shows that foraminifera collected at each transect might actually have travelled long distances within the 30 days prior to sampling at the sample locations. The length of the modelled trajectories varies greatly from location to location, ranging between 200-500km. This resulted in a variability (SD) within one transect ranging from 0.11 to 1.0°C and 0.03 to 0.4 salinity units.

## DISCUSSION

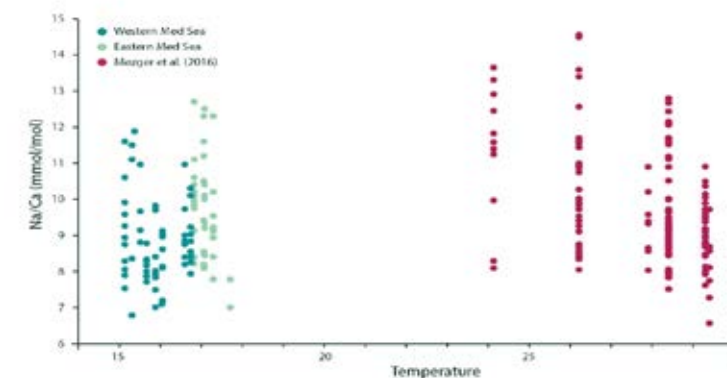
### SALINITY, $\delta^{18}\text{O}$ AND $\delta\text{D}$ OF THE SEA WATER

A single uniform and stable trend in sea water stable isotopes with salinity is a prerequisite for reconstructing past salinities. This is important not only when using the stable oxygen isotopes measured on foraminiferal shell carbonates, but also for the interpretation of the hydrogen isotopic composition of alkenones, which are also used as proxies for paleo-salinity (Schouten et al., 2006; Vasiliev et al., 2013; Weiss et al., 2017).

The data presented here substantially increases the amount of data on the relation between salinity and water isotopes of the Mediterranean (Fig. 2). Although the new data clearly overlap with existing data, we also observe slight but statistically significant differences in the average salinity to  $\delta^{18}\text{O}$  relationship for the different data sets. The overall lower  $\delta^{18}\text{O}$  values of sea water measured here compared to the combined set of surface sea water isotopes from Stahl and Rinow (1973), Pierre et al. (1986), Gat et al. (1996), Pierre (1999) and Cox (2010) of approximately 0.3‰ (Fig. 2) may be explained by inter-decadal, seasonal and geographical variability between sample sets, or a combination of these factors. Importantly such offsets also give a first order indication of the limit to the accuracy and precision of reconstructions of past salinity using a combined temperature-stable isotope approach from the primary relationship used.

Although Gat et al. (1996) reported a markedly different  $\delta\text{D}/\delta^{18}\text{O}$  relationship for the Eastern Mediterranean Sea compared to that of the Western Mediterranean Sea, our results show no sign of such a longitudinal discontinuity for the same area (Fig. 2). This implies that the water isotopic composition of the entire Mediterranean Sea can primarily be described by a single mixing line between two endmembers, with high versus lower salinity, respectively. The remarkable trend between  $\delta\text{D}/\delta^{18}\text{O}$  observed previously by Gat et al. (1996) was explained as a deuterium excess effect due to a combination of the composition of the lowermost air vapor and mixing with the enriched

surface waters, most notable in winter months. The discrepancy in  $\delta\text{D}/\delta^{18}\text{O}$  relationship observed between our data and those of Gat et al. (1996) may be due to inter-decadal variability in the hydrological cycle or by differences in seasonal coverage. Potentially the observations of Gat et al. (1996) were hence either related to unusual conditions, spatially restricted features not covered by our sampling locations or the hydrological cycle in the eastern Mediterranean has recently changed considerably. Either way the observed offset between the western and the eastern basin is apparently not stable and should therefore probably not be considered when using Mediterranean stable isotope signatures for reconstructing paleo-salinities.



**Figure 5.** The ratio of Na/Ca in *G. ruber albus* appears to be independent from sea water temperature.

While Mezger et al. (2016) showed a negative relationship between temperature and foraminiferal Na/Ca in specimens collected from the Red Sea, the addition of new data from the Mediterranean Sea shows clearly that the previously hypothesized negative impact of temperature on Na/Ca is likely an artefact of the negative relationship of temperature and salinity in the Red Sea and that temperature has no significant impact on Na/Ca

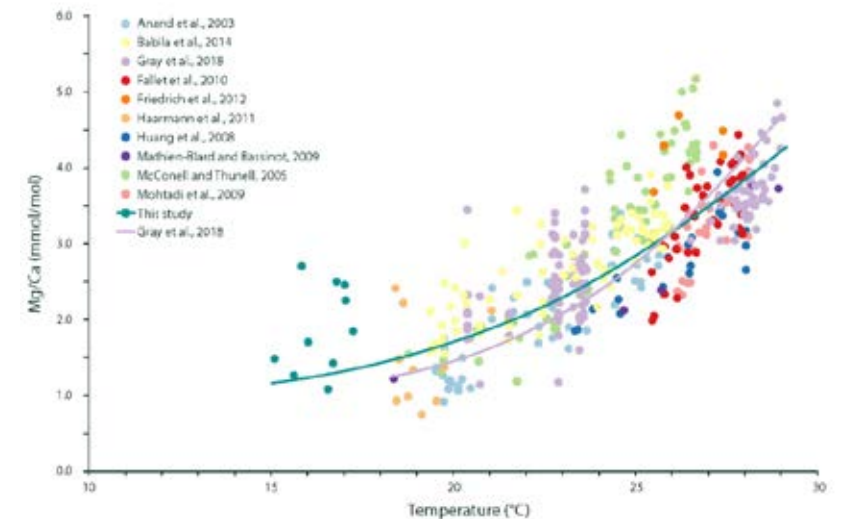
### NA/CA VS SALINITY

The Na/Ca ratios measured on the carbonate shells of *G. ruber albus* from the Mediterranean Sea are significantly and linearly correlated to salinity (Fig. 4 a). This relationship is similar to the one reported previously for plankton pump-collected *G. ruber albus* from the Red Sea (Mezger et al., 2016). Mezger et al. (2016) suggested that there might a combined effect of different environmental factors such as carbonate chemistry, salinity, and temperature on the Na/Ca values in the field-collected specimens. In the Red Sea it is not possible to decouple these factors as they are strongly related. Since in contrast to the Red Sea where there is a strong negative correlation between salinity and temperature, the Mediterranean Sea surface salinity and temperature are positively correlated to each other, comparing our data to that of Mezger et al.

(2016) allows to decouple temperature from salinity (Fig. 5). This shows that the correlation between foraminiferal Na/Ca values and temperature observed in the Red Sea was not causal and more likely caused by salinity (Mezger et al. 2016). If temperature would have a significant effect on the Na/Ca values in *G. ruber albus*, we would expect different slopes and/or offsets for the Na/Ca to salinity calibrations for the Mediterranean Sea and Red Sea. This implies that temperature has no or only a minor impact on Na/Ca ratios in *G. ruber albus* shells, which is in line with similar findings showing a lack of temperature effect on the Na/Ca of *T. sacculifer* (Bertlich et al., 2018). The average standard deviation in Na/Ca values for a given salinity corresponds approximately to 2 salinity units, using the calibration given here (Fig. 4 a). This large variability is similar to the inter-chamber and inter-specimen variability in other El/Ca ratios, such as for example in Mg/Ca reported by Sadekov et al. (2008) and appears to be inherent to single-chamber El/Ca (de Nooijer et al., 2014b). It has been suggested that such variability between individuals and also between different chambers of the same individual, may be caused by differences in living depth (and hence environmental conditions (Mezger et al., 2018)), lateral transport (van Sebille et al., 2015) or variability in element incorporation during biomineralization due to vital effects (Erez, 2003; de Nooijer et al., 2014a; Spero et al., 2015) or individual timing of chamber formation (Dämmer et al., 2019). Since specimens used here were collected from surface waters and add new chambers very frequently, vertical or literal migration into waters with significantly different conditions as suggested by Mezger et al. (2018) and Van Sebille et al. (2015) appears to be an unlikely cause for heterogeneity between specimens in this case. The relatively large scatter in Na/Ca values observed for single chambers (Fig. 4a) implies that accurate and precise reconstructions of salinity can only be based on combining a substantial number of specimens (Wit et al., 2013).

If salinity is reconstructed from the Na/Ca measurements using the calibration published by Mezger et al. (2016) and compared versus salinity measured in situ in the Mediterranean Sea, the reconstructed salinity follows the in situ measurements closely almost 1:1. The largest deviation from this 1:1 relationship occurs in the lower salinity range, at a salinity of 36.52 the reconstructed salinity estimates underestimate salinity by 0.71 salinity units. The average difference between in situ salinity measurements and salinity reconstructed based on one single-chamber measurement is an underestimation of salinity by 0.46 salinity units. This is still higher than the theoretical uncertainty associated when combining foraminiferal  $\delta^{18}\text{O}$  and temperatures derived from Mg/Ca measured at exactly the same specimens (Rohling, 2007). An uncertainty (1SD) of 1 °C in the Mg/Ca-temperature calibration (which may be particularly optimistic at high seawater temperatures), would result in an uncertainty of ~0.37 units for the reconstructed

difference between two salinities. This approach will lead to an improved salinity reconstruction when the (change in) past temperatures are determined more precisely, for example by reducing the error through increased sample size. The same applies for salinity reconstructions based on Na/Ca, for which not many calibrations are available and hence, leaves room for improvement.



**Figure 6.** The relationship between Mg/Ca in *G. ruber albus* and temperature during calcification

can be described using the following exponential equation:  $\text{Mg/Ca} = 0.278 * \exp(0.093 * T)$  for a temperature range from 15.1 to 29.1 °C.

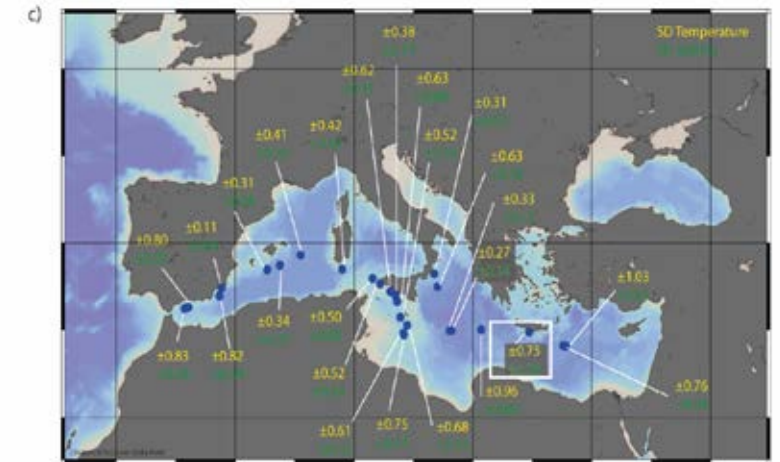
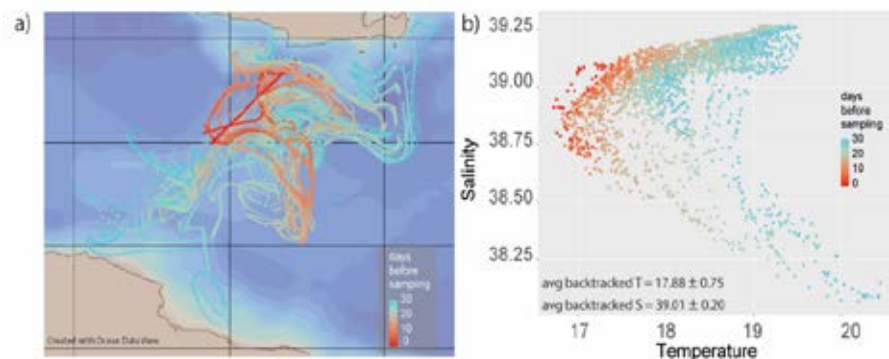
While these reconstructions as well as the lack of a strong temperature effect are very encouraging results for the use of Na/Ca as a salinity proxy, the incorporation of Na into foraminiferal calcite does not appear to be homogenous across the entire shell. It has been shown that the majority of Na in *G. ruber albus* is located in the spines (Mezger et al., 2018, 2019), which are not well preserved in the fossil record.

## G. RUBER ALBUS MG/CA VALUES

The increase in Mg/Ca in *G. ruber albus* with temperature (Fig. 4b) fits recent calibration efforts for Mg-incorporation and temperature (e.g. Gray et al., 2018). Since salinity and inorganic carbon chemistry also both affect Mg incorporation in this species (Kisakürek et al., 2008; Gray et al., 2018), and the Mediterranean exhibits large gradients in these parameters, it is necessary to correct measured Mg/Ca values for these

parameters. After normalizing Mg/Ca values to a sea water salinity of 35, using the calibration of Gray et al. (2018), the dependency of the Mg/Ca on temperature is similar to previously reported calibrations (e.g., Gray et al., 2018), although the Mg/Ca values at the lower most temperatures appear to be higher than expected (Fig. 6). This could potentially be caused by a combination of an underestimation of the salinity effect in these highly saline waters, since salinities observed here are well outside the calibration range used by Gray et al. (2018), and low temperatures, impacting the foraminiferal Mg/Ca comparatively little.

Adding our results to published Mg/Ca-temperature-calibrations for *G. ruber albus* (Anand et al., 2003; Babila et al., 2014; Fallet et al., 2010; Friedrich et al., 2012; Gray et al., 2018; Haarmann et al., 2011; Huang et al., 2008; Kisakürek et al., 2008; Mathien-Blard and Bassinot, 2009; McConnell and Thunell, 2005; Mohtadi et al., 2009) now extends the combined calibration to lower temperatures (i.e. < 18°C), maintaining a comparatively low temperature sensitivity in the colder part of the calibration (Fig. 6). This not only increases confidence in the application of Mg/Ca in this species as a paleotemperature reconstruction tool for colder temperatures, but also support application of individual foraminiferal Mg/Ca values for reconstructing seasonality (Wit et al., 2010). Although low densities were reported previously for *G. ruber albus* in the Mediterranean Sea during winter time, including being absent in large areas (Pujol and Grazzini, 1995; Bárcena et al., 2004) our finding implies that lowest values in Mg/Ca can be related to winter temperatures. *G. ruber albus* is not only present throughout the year as also shown by Rigual-Hernández et al. (2012) and Avnaim-Katav et al. (2020), but it also registers the in-situ temperature, also during seasons which are close to its lower temperature limit. Admittedly the large scatter also observed at one single sampling time (i.e. season) makes the deconvolution of seasonality from analyzing single specimen Mg/Ca values challenging.



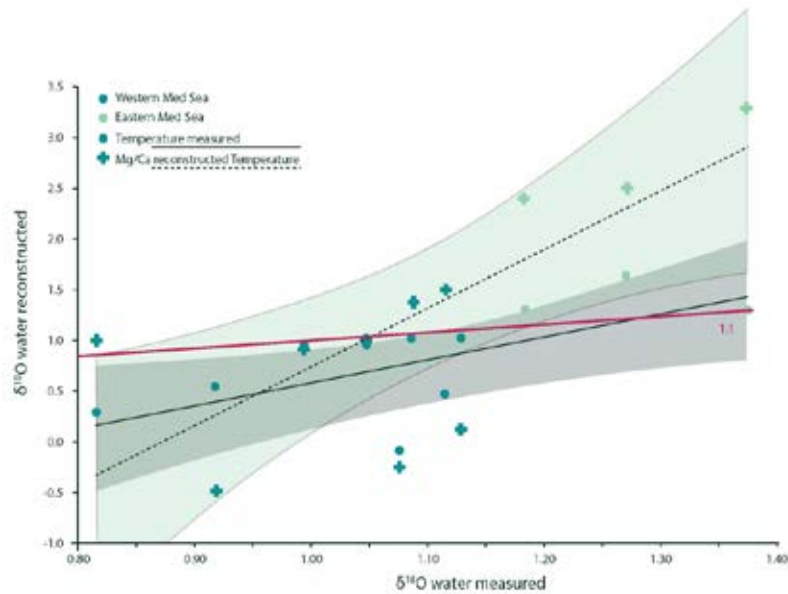
**Figure 7.** (a) Example of back-tracked pathways for a single transect (the one marked by a white rectangle in panel c). The colour indicates the time before sampling up to 30 days. (b) Analysing the different environmental conditions at the different locations of these potential paths show that foraminifera sampled very likely experienced a large range in temperatures as well as salinities. (c) The variability in potentially experienced environmental conditions varies considerably from location to location, as indicated by notation of 1 standard deviation for both parameters for each sampling location. Maps in (a) and (c) were generated using Ocean Data View version 4.

$\delta^{18}\text{O}_{\text{FORAMINIFER}}$

## ROLE OF LATERAL TRANSPORT ON $\delta^{18}\text{O}_{\text{FORAMINIFER}}$

Horizontal transport of planktonic foraminifera may increase exposure to variable environmental conditions, including different temperatures, salinities and seawater stable isotope compositions (van Sebille et al., 2015). Comparing the sampled transects with the calculated back tracking trajectories shows that especially close to the straits (Alboran Sea and Strait of Sicily) the area where the foraminifera might be derived from, potentially extends over considerable distances and therefore, variability in environmental parameters. With the surface variability in temperature and salinity during the sampling period, the calculated variability in these parameters varied between 0.11 and 1.03°C per transect and 0.04 and 0.39 salinity units per transect (Fig. 7b, c). This means that the majority of foraminifera experienced a variability of approximately 0.5°C and 0.15 salinity units.





**Figure 8.** The relationship between  $\delta^{18}\text{O}_{\text{seawater}}$  measured in the Mediterranean Sea and  $\delta^{18}\text{O}_{\text{seawater}}$  calculated from foraminiferal geochemistry (*G. ruber albus*). The relationship shown with dashed lines and cross shaped markers represents values calculated using foraminiferal  $\delta^{18}\text{O}$  as well as Mg/Ca as an additional temperature proxy to decouple the effect of temperature and salinity on  $\delta^{18}\text{O}$ . The relationship shown with the continuous lines and circular markers shows the same samples, but instead of using temperature values derived from foraminiferal Mg/Ca ratios, in situ measurements for temperature were used, the relationship can be described as  $\delta^{18}\text{O}_{\text{water_reconstructed}} = 2.62 (\pm 0.69) * \delta^{18}\text{O}_{\text{water_measured}} - 63.99 (\pm 26.11)$  with an adjusted  $R^2$  of 0.37. The temperature gradient was  $2.2^\circ\text{C}$ .

When considering calibrations, this is not affecting the measured proxy variables as the difference may be unbiased but adds to the uncertainty of the environmental parameter to be reconstructed. Since foraminifera grow by periodically adding chambers and since the size of the added chambers increases exponentially in many species, the carbonate added closer to the sampling location makes up a larger proportion of the total shell mass than carbonate added at earlier life stages. Therefore, chambers formed early during a foraminifer's life, have less impact on average shell composition and hence the calibration and the back tracking trajectories (Fig. 7a-c) thus indicate the largest possible range of conditions experienced by a single foraminifer. This is relevant when considering whole-shell chemistry (i.e., oxygen isotopes; Fig. 4 a) and to a lesser extent also when considering the elemental composition of the final chamber (Fig. 4 b). The last chamber is affected by a much smaller range in environmental conditions, i.e., only the timespan during which the final chamber was built, not

more than a few days prior to sampling.

Since  $\delta^{18}\text{O}$  of the calcite could not be measured on F-chambers only, like for element/Ca ratios, and several specimens were needed for a single analysis, results reflect average composition of foraminiferal populations at the sampling areas. The averaging effectively cancels out differences due to inter- and intra-individual variability, but not offsets due to lateral transport. When transport directions are largely uniform, this results in biases and should not add to the scatter in the calcite's isotope composition. Hence this transport affects the calibration but does not affect precision.

## IMPLICATIONS FOR PROXIES

Combining existing calibrations for foraminiferal Mg/Ca and temperature (Gray et al., 2018) and calibrations relating  $\delta^{18}\text{O}_{\text{foraminifera}}$  with temperature (Mulitza et al., 2003), the  $\delta^{18}\text{O}_{\text{seawater}}$  can be calculated. With our dataset we here assess the quality of such reconstructions by comparison to measured  $\delta^{18}\text{O}_{\text{seawater}}$  (Fig. 8). The Mg/Ca values used here were not corrected for salinity effects, since salinity is the target parameter that has to be reconstructed and is thus treated as unknown. Even though there is a carbonate ion effect on the Mg/Ca in *G. ruber albus* (Evans et al., 2016; Gray et al., 2018; Kisakürek et al., 2008), the measured values were not corrected for this, since this factor is also unknown in paleo-reconstructions.

Calculated and measured  $\delta^{18}\text{O}_{\text{seawater}}$  do not follow a 1:1 correspondence which could be caused by uncertainties in the different proxy calibrations, analytical uncertainties, heterogeneous element and isotope composition within and between specimens, variability in the location and timing of their calcification and the effect of salinity and pH on Mg/Ca. The lack of a strong correlation between calculated and measured  $\delta^{18}\text{O}_{\text{seawater}}$  in our dataset implies that calculating salinity from reconstructed  $\delta^{18}\text{O}_{\text{seawater}}$  values will not yield meaningful salinity reconstructions, since reconstructed values for  $\delta^{18}\text{O}_{\text{seawater}}$  are not well correlated to in situ measured  $\delta^{18}\text{O}_{\text{seawater}}$ . Calculating salinities from  $\delta^{18}\text{O}_{\text{seawater}}$  clearly adds much uncertainty due to spatial and temporal variability in the correlation of these two parameters (Conroy et al., 2017; LeGrande and Schmidt, 2006; McConnell et al., 2009).

It is important to note that the scatter in the foraminiferal chemistry can only to a small degree be explained by lateral transport (Fig. 7). This effect may be larger in areas where the environmental conditions vary more strongly over the distance travelled by the foraminifer, and/or in basins where there is simply more lateral transport over



the foraminifer's lifetime. In our exercise, the calculated trajectories add only a minor component to the uncertainty in T (often within 0.75 °C; Fig. 7) and salinity (often within 0.25 salinity units).

In our dataset, the uncertainty in salinity estimates based on  $\delta^{18}\text{O}_{\text{seawater}}$  is much smaller when using in situ measured temperatures (Fig. 8). The sum of squares of the residuals (difference between reconstructed and measured values) is 9.04 when using temperatures derived from Mg/Ca and  $\delta^{18}\text{O}_{\text{foraminifera}}$ , but only 3.56 when using temperatures measured in situ, indicating a better reconstruction.

This shows that the uncertainty or offset in temperatures derived from Mg/Ca, even though the Mg/Ca-temperature relationship is studied relatively extensively for *G. ruber albus*, is most likely the most limiting step. Even though in our dataset temperatures reconstructed from Mg/Ca deviated less than 2°C from the measured temperature, these small offsets have a large effect on the reconstructed  $\delta^{18}\text{O}_{\text{seawater}}$ . It is therefore crucial to choose temperature proxies carefully, use a large enough number of specimens for analysis, be aware about potential effects of lateral particle transport as well as other environmental parameters, and to be conscious about how errors propagate in paleoclimate reconstructions.

Combining all foraminiferal shell chemistry results show that salinities based on  $\delta^{18}\text{O}$  and Mg/Ca may under some specific conditions allow calculating past salinity, but the uncertainties in  $\delta^{18}\text{O}_{\text{seawater}}$  are large even in a setting with a large salinity gradient such as the Mediterranean Sea. This is in line with predictions of uncertainty based on theoretical considerations (Rohling, 2007). The most limiting step in these calculations is the reconstruction of past temperatures, which should be better than 2 degrees. The development, validation, and improvement of other, more direct salinity proxies such as foraminiferal Na/Ca therefore remains crucial for more reliable paleo-salinity reconstructions.

## CONCLUSION

Using plankton pump samples from the Mediterranean Sea, we showed that 1) the relationship of Mg/Ca in *G. ruber albus* and sea water temperature at lower temperatures follows an exponential relationship, therefore the proxy can now also be applied to lower temperature ranges (<18°C) than before, covering almost the entire temperature tolerance range of that species, though sensitivity of the calibration is comparatively low at low temperatures, 2) the combination of foraminiferal  $\delta^{18}\text{O}$  and Mg/Ca together

with assumptions about  $\delta^{18}\text{O}_{\text{seawater}}$  values and  $\delta^{18}\text{O}_{\text{seawater}}$  – salinity relationships does not lead to useful reconstructions of seawater salinity 3) foraminiferal Na/Ca correlates well with sea surface salinity and is independent from temperature, making it a potentially valuable tool for salinity reconstructions.

## DATA AVAILABILITY

The data on which this manuscript is based are available at the 4TU.Centre for Research Data (doi: 10.4121/uuid:25aa0dc8-2ce2-44e9-acf3-a669f4439932).

## AUTHOR CONTRIBUTION

LKD, LdN and GJR designed the study and performed the sample collection. LKD and JGH prepared and processed the samples and the corresponding data. EvS performed the particle backtracking. All authors were involved in data interpretation. LKD drafted the manuscript with contributions from all authors.

## ACKNOWLEDGEMENTS

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# CHAPTER SIX

## 15,000 YEARS OF HYDROLOGICAL CONDITIONS IN THE WESTERN MEDITERRANEAN SEA INFERRED FROM FORAMINIFERAL TRACE METAL PROFILES

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Lennart de Nooijer and Gert-Jan Reichart

### ABSTRACT

There is still large uncertainty in the future response of the circulation in the Mediterranean Sea to climate change. Past changes in Mediterranean circulation, such as related to Holocene sapropel formation in the Eastern Mediterranean basin (~10.5 – 6.1 ka BP), can assist to shed light on the relationship between hydrological conditions (i.e., salinity and temperature) and circulation changes in the surface and deep waters of both the Eastern and Western Mediterranean. In this study we assess this relationship between changing hydrological conditions and circulation over the past 15,000 years based on foraminiferal (benthic and planktonic) shell chemistry from the Iberian margin. Results show that manganese, mobilized through low oxygen concentrations during the interval of sapropel S1 deposition in the eastern basin, must have been transported to the Western Mediterranean. This is indicated by elevated Mn lev-

els in benthic deep-sea foraminifera, while planktonic foraminifera show stable levels over the same time period. Therefore, the Western and Eastern Mediterranean basins must have maintained a connection that allowed the transport of dissolved Mn throughout the entire basin in the deep sea, which is in line with previous studies suggesting a continued anti-estuarine circulation in the Mediterranean albeit less vigorously. We show that the biogeochemical signals of the water masses originating from the Eastern Mediterranean reached far into the Western Mediterranean during the first stage of the sapropel S1 formation. This suggests that during those times the Eastern Mediterranean may have contributed in maintaining relatively low oxygen conditions, as expressed by the so-called Organic-Rich Layers, in large parts of the Western Mediterranean.

## INTRODUCTION

The Mediterranean Sea is a semi-enclosed basin, with surface water inflow from the Atlantic Ocean via the Strait of Gibraltar. This Atlantic inflow compensates the excess evaporation over freshwater supply within the Mediterranean basin (e.g. Pinardi & Masetti, 2000), and while traversing from the Western to the Eastern Mediterranean the density of this Atlantic water increases, forming the so-called Modified Atlantic Water (MAW). In cold winters, the intense air-sea interactions can lead to downwelling of the saline MAW in the Levantine basin, which then forms the Levantine Intermediate Water (LIW), settling to a depth between 200 and 600 meters (Wüst, 1961). This water mass spreads over the entire Mediterranean basin (often referred to as Mediterranean Intermediate Water in the Western Mediterranean) and contributes to the Mediterranean Outflow Water (MOW) flowing back into the Atlantic. Formation of the LIW is highly sensitive to monsoonal changes, mainly through changes in freshwater supply by the Nile River (Bahr et al., 2015; Rossignol-Strick et al., 1982). Mixing of LIW with colder but less saline waters causes the formation of deep water in both the Western and Eastern Mediterranean. This occurs in the Gulf of Lion for the Western Mediterranean (i.e., Western Mediterranean Deep Water; WMDW) and the Adriatic- and Aegean Seas in the Eastern Mediterranean Sea (i.e., Eastern Mediterranean Deep Water; EMDW) (Pinardi and Masetti, 2000). A simplified schematic of the Mediterranean Sea circulation patterns can be found in Fig. 1.

The Western as well as Eastern Mediterranean Sea experienced major changes in hydrological conditions on geological time scales (Rohling et al., 2015). The Eastern Mediterranean palaeoceanographic evolution is punctuated by rapid and large changes in circulation and biological productivity, resulting in anoxia and the deposition of so-called sapropels (Rossignol-Strick et al., 1982). These sapropels are characterized by

high organic carbon contents generally over 1-2 mass% (Kidd et al., 1978; Murat and Got, 2000), signifying major rearrangements in surface and deep-water circulation and/or export productivity. Sapropels are generally more common in the Eastern Mediterranean while in the Western Mediterranean Sea, Plio-Pleistocene sapropels have generally low organic carbon contents up to at most 3% (Murat, 1999). These western equivalent of the sapropels are being referred to as Organic Rich Layers (ORL's), as in many cases they contain less than 2 % organic carbon (Rogerson et al., 2008). The link between Eastern Mediterranean sapropels and Western Mediterranean ORL is complex. For instance, the most recent sapropel (S1) is deposited between ~10.5 and 6.1 kyr BP (Grant et al., 2016; De Lange et al., 2008), while the last ORL is slightly older (between 14.5 and 8.2 kyr BP; Rogerson et al., 2008). This reflects how the different basins respond to changes in ventilation and productivity, but the exact connection between these events remains eluded.

The occurrence of sapropels in the Eastern Mediterranean has been linked to changes in past freshwater input by the river Nile (Rossignol-Strick, 1985; Rossignol-Strick et al., 1982) or from the northern border lands of the basin (Filippidi and De Lange, 2019; Rohling and Hilgen, 1991). The enhanced input of freshwater would have stimulated productivity by the added nutrients and altered water column overturning by changing stratification (Emeis et al., 2003; Rohling et al., 2015). Although early studies (e.g. Thunell & Williams, 1989) suggested a complete reversal in circulation might have occurred (i.e., a shift from anti-estuarine to estuarine), it is now generally assumed that circulation in the Eastern Mediterranean primarily slowed down significantly (Rohling and De Rijk, 1999; Sweere et al., 2021). In combination with enhanced productivity, this likely resulted in the water column becoming dysoxic to anoxic or even euxinic (Rohling et al., 2006). The low oxygen concentrations in the water column impacted local metal geochemistry and especially manganese was remobilized from the sediment escaping to the water column (De Lange et al., 2008). To what extent the altered Eastern Mediterranean metal cycling also affected the Western basin and thus how much eastern conditions may have influenced the west, is yet unknown. The interaction between sea-level effects, melt-water spills into the North Atlantic, Alpine meltwater generation, and monsoon forcing may have further affected changes in past Western Mediterranean circulation (see Rohling et al., 2015).

Here we present a high-resolution record from the south-eastern Iberian margin on the interface between the Algerian Basin and the Alboran Sea to reconstruct changes in water circulation during the Holocene. This record contains the time interval corresponding to the deposition of sapropel S1 in the Eastern, and ORL 1 in the



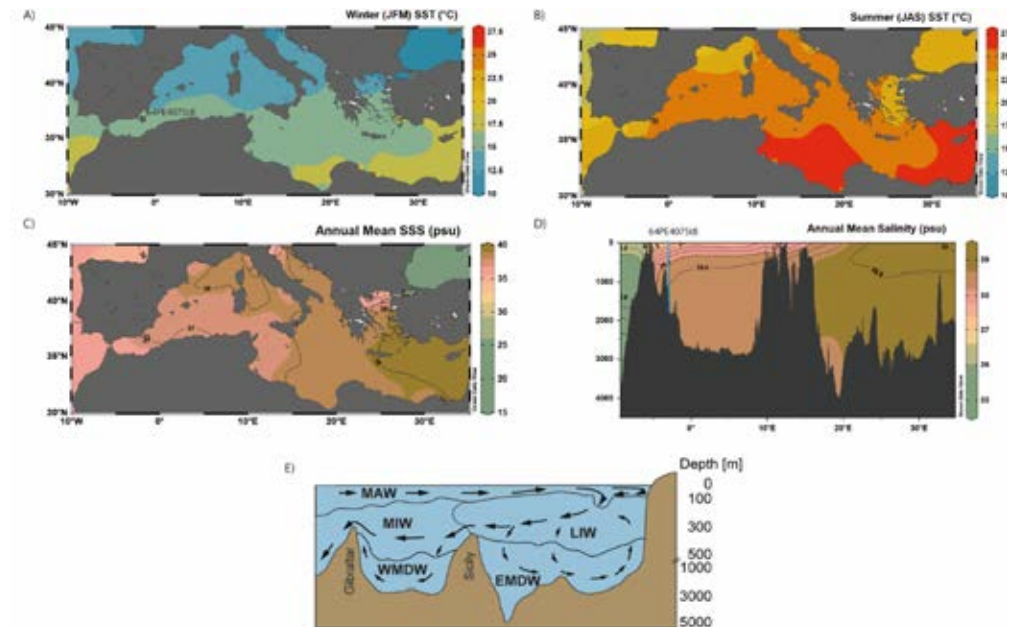
Western Mediterranean Sea, both reflecting changes in circulation strength and productivity. We assess changes in hydrological conditions (i.e., salinity and temperature) at the surface and deep water using planktonic and benthic foraminiferal proxies, respectively. Changes in intermediate water flow strength have been assessed previously (Bahr et al., 2015; Toucanne et al., 2012) and our study allows to cross compare these data to salinity/temperature gradient changes in the Western Mediterranean. As changes in monsoon intensity resulted in the Eastern Mediterranean sapropels, we will also investigate the results of the processes responsible for the formation of sapropels also in the Western basin, looking at the potential transport of remobilized Mn across the basin. Although Mn is very sensitive to oxygen levels and mobilized easily under oxygen depleted condition (Middelburg et al., 1987) the rather slow kinetics of Mn oxidation allow dissolved Mn to travel thousands of miles from its origin before being removed from the water column (Saager et al., 1989). Elevated Mn levels in the water column might be reflected by a record of foraminiferal Mn/Ca (Barras et al., 2018) which will be complemented by a temperature reconstruction of both surface and bottom water, as well as a reconstruction of redox conditions based on X-ray Fluorescence (XRF) core scanning of the sediments.

## MATERIALS AND METHODS

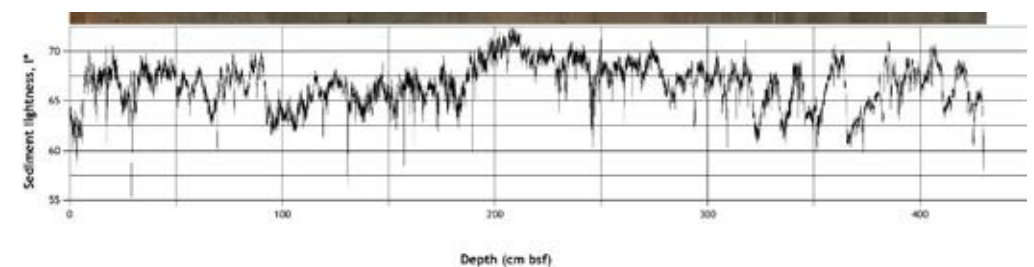
### LOCATION

The sediment core 64PE407St8 (1995 meters depth; bathing in WMDW) was collected by piston coring from Station 8 (36°44.99'N, 01°29.99'W, Fig. 1) during a cruise in the Mediterranean Sea in 2016 (64PE407 NESSC West Med, RV *Pelagia*) from the Iberian Margin (Reichart et al., 2023). The station was located on the slope, but the exact location was selected because the area appeared relatively flat in the multibeam bathymetry. This location is in the transition zone between the Alboran and Balearic Seas.

The 8.31 m long sediment core was opened, and the sedimentary sequence was described. Subsequently, detailed line-scan pictures were taken from the core sections, after which the sections were analysed for their bulk elemental composition using an XRF core scanner. The sediments recovered consist of hemipelagic mud, brownish grey coloured, with small black spots. The sediments show subtle changes in colour down-core on a decimetre scale (Fig. 2). Throughout the core small shell fragments are observed, without any indications of changes in overall sedimentology in at least the top 4.3 meters which is the target interval of this study.



**Figure 1.** (A) Mean winter sea surface temperature, (B) mean summer sea surface temperature, (C) annual mean sea surface salinity in the Mediterranean Sea, (D) salinity profile and (E) a simplified schematic of the Mediterranean Sea circulation. Coring location is marked with a star. Temperature and salinity data were taken from the World Ocean Atlas 2018 (Boyer et al., 2018), figures 1A-D were created using Ocean Data View (Schlitzer, 2018) with custom colour palettes (Raftar, 2019).



**Figure 2.** Sediment lightness ( $l^*$ ) plotted versus depth of the top 431cm of the core (core sections 9-5). The top part of the figure is based on a line scan of the sections.



## SEDIMENT GEOCHEMISTRY

Bulk geochemical sediment composition was analysed using an Avaatech core scanner at the Royal Netherlands Institute of Sea Research (NIOZ). The core was scanned at 10, 30, and 50kV with a 10-mm resolution (slit size 10 by 12 mm). We here focus on barium (Ba; as export-productivity proxy; e.g. Bishop, 1988) and bromine (Br; as proxy for marine organic carbon; e.g., Ziegler et al., 2008). To calibrate (using Avaaxelerate; Bloemsma, 2015) and verify the XRF-scanning signals, we also performed low-resolution (28 samples) inductively coupled plasma mass spectrometry (ICP-MS) measurements on totally destructed samples. Repeated analyses of standards (MESS-3) showed an accuracy better than 10% and precision better than 3% for all target elements. These ICP-MS results allow to also use Uranium (U) as a proxy for sub-oxic conditions and Molybdenum (Mo) as proxy for the presence (or lack of) euxinic conditions (e.g. Algeo & Tribovillard, 2009). The Ba from XRF-scanning was calibrated with the Multivariate Log-ratio approach (Bloemsma, 2015), while for Br we normalized on total counts to compensate for difference in sediment density (as we did not produce any Br data through ICP-MS).

## STABLE ISOTOPE ANALYSES

Analysis on foraminifera was only performed on samples from the top half of the core (top 4.31m) since we here target the time frame during S1 deposition. Therefore, oxygen isotopes were measured on *Globigerina bulloides*, picked from the top 0.31 m at a 5 cm resolution, at a 1 cm resolution for core section 0.31 to 1.31 m and at a 10 cm resolution from 1.31 to 4.31 m downcore. Stable carbon and oxygen isotopes of their carbonate shells were measured using an automated carbonate device (Kiel IV, Thermo Scientific) coupled online to a Thermo Finnigan MAT 253 dual-inlet isotope ratio mass spectrometer (IR-MS). Data is reported with respect to the VPDB standard with standard deviation and precision always being within 0.1 ‰ and 0.07 ‰ for oxygen ( $\delta^{18}\text{O}$ ) and carbon ( $\delta^{13}\text{C}$ ) isotopes respectively, based on international (NBS 19 limestone) and in-house standards (NFHS-1, (Boer et al., 2022; Mezger et al., 2016))

## FORAMINIFERAL GEOCHEMISTRY

During the same cruise in which the sediment core was retrieved, planktonic foraminifera were collected from the Western Mediterranean Sea surface waters (Dämmer et al., 2020) using a so-called plankton pump (Ottens, 1992) during 6 hour long

transects. From these samples, *Globigerinoides ruber albus* were previously analysed to investigate the combination of Mg/Ca and  $\delta^{18}\text{O}$  to reconstruct past sea water temperature and salinity (Dämmer et al., 2020). Here, we analysed specimens of *Globorotalia inflata* from a selection of the same set of samples to assess the potential of this species in reconstructing seawater temperatures from mid-depth. Plankton pump derived samples where freeze dried and ashed at a low temperature to remove organic remains; sample preparation is described in detail by Dämmer et al. (2020).

Benthic foraminifera were picked from the top 1.61m of the core for element analysis of their carbonate shells from a selection of the same samples as described for  $\delta^{18}\text{O}$  measurements on *G. bulloides* (see section ‘Stable isotope analyses’ above). Specimens of the benthic species *Cibicidoides wuellerstorfi*, *Gyroidina altiformis* and the aragonitic *Hoeglundina elegans* were used, though abundances were sometimes very limited. *Cibicidoides wuellerstorfi* for example did not occur in the S1 time interval, a pattern that has previously been observed in the Eastern Mediterranean Sea (e.g., Abu-Zied et al., 2008), and analysis results for this species are therefore omitted. Specimens were all cleaned according to the Barker et al. (2003) cleaning protocol with a reductive cleaning step added, using hydrazine (see e.g. Boyle & Keigwin, 1985).

Individual benthic specimens were measured by laser ablation quadrupole inductively coupled plasma mass spectrometry (LA-Q-ICP-MS) using a circular spot with a laser diameter of 80  $\mu\text{m}$ . The laser system (NWR193UC, New Wave Research) at NIOZ was used in combination with a two-volume sample cell (TV2). An energy density of  $1 \pm 0.1 \text{ J cm}^{-2}$  and a repetition rate of 6 Hz were used for ablation in a helium environment. The resulting aerosol was transported by a  $0.7 \text{ L m}^{-1}$  helium flow to an in-house custom-built smoothing device (Steinhardt et al., 2015) before entering the quadrupole ICP-MS (iCAP Q, ThermoFisher Scientific). Masses of  $^{11}\text{B}$ ,  $^{23}\text{Na}$ ,  $^{27}\text{Al}$ ,  $^{25}\text{Mg}$ ,  $^{43}\text{Ca}$ ,  $^{44}\text{Ca}$ ,  $^{55}\text{Mn}$ ,  $^{88}\text{Sr}$  and  $^{137}\text{Ba}$  were monitored, with  $^{44}\text{Ca}$  serving as an internal standard. Standards NIST610, NIST612, BAMRS3, MACS3, JCp1 and JCt1 were used, with NFHS-2-NP (Boer et al., 2022) used for drift correction. Average accuracy was 98% and the relative standard error was 2.8 and 4.0 for  $^{25}\text{Mg}$  and  $^{55}\text{Mn}$  respectively.

Specimens of the planktonic foraminifers *Globigerinoides ruber ruber* (Morard et al., 2019; syn. *G. ruber* (pink)) and *G. inflata* were picked from the same samples as used for oxygen isotope measurements on *G. bulloides* (see section ‘Stable isotope analyses’ above) where possible. All samples were cleaned according to Barker et al. (2003) with a reductive cleaning step added, using hydrazine (see e.g., Boyle & Keigwin, 1985).

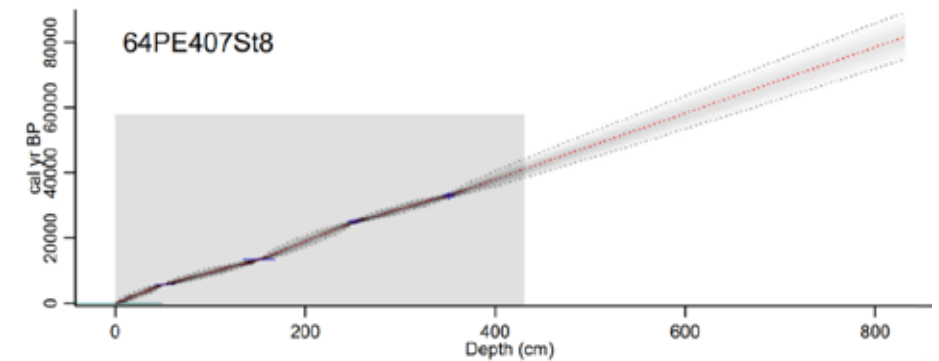
Specimens from each sample were pooled prior to analysis and dissolved in 0.5 ml 0.1 M HNO<sub>3</sub>. A pre-scan for [Ca] was performed on an Element 2 sector field double focusing mass spectrometer (SF-ICP-MS) to allow for a relatively uniform matrix composition across samples. Measurements included masses <sup>23</sup>Na, <sup>25</sup>Mg, <sup>27</sup>Al, <sup>43</sup>Ca, <sup>55</sup>Mn, <sup>88</sup>Sr, <sup>137</sup>Ba and <sup>238</sup>U; all element counts were converted to Ca-ratios assuming an abundance of 40% Ca in the foraminiferal shells. Samples were measured against Carbonate standards JCp-1 and JcT-1 using NFHS-2-NP (Boer et al., 2022) for drift correction. Accuracy was better than 95%.

## AGE MODEL

The age model was constructed using accelerator mass spectrometry (AMS) <sup>14</sup>C dating of four samples, using a mixture of planktonic foraminifera. The <sup>14</sup>C AMS measurements were performed at the Poznan radiocarbon laboratory in Poland (Table 1). The R software package ‘Bacon’ (Blaauw and Christen, 2011) was used to generate a Bayesian deposition model using the radiocarbon ages (Table 1) and their corresponding uncertainties (Fig. 3). Age model parameters were chosen based on the recommendations in Blaauw & Christen (2011) for an environment with low “memory” in accumulation rates (i.e., acc. shape=2, mem. strength=20, mem.mean=0.1). The Marine13 curve was used for calibration of our radiocarbon-dated samples by running the ‘Bacon’-script. As little deviation from the global marine carbon calibration curve is expected, we did not apply an additional correction for potential local deviations ( $\Delta R$ ) from the global average marine reservoir age. The age model (Fig. 3) shows that sedimentation rate was rather uniform throughout the sequence (~0.1 mm/year). The older part of the record must be regarded with caution and considerable offsets may occur due to the distribution of the dated samples only in the upper half of the core. However, here we concentrate on the last 25 kyr only, with a particular focus on the Holocene. The slightly steeper depth versus age curve at the core top suggests that the uppermost part of the record may have been lost during coring, which is a well-known issue with piston coring.

**Table 1.** <sup>14</sup>C ages and corresponding depths of mixed planktonic foraminifera collected from the sediment core, and uncertainties in the ages. Sample identifiers refer to the lab codes used in the Poznan AMS <sup>14</sup>C laboratory.

Depth in core [cm]	<sup>14</sup> C Age	Sample identifier
51.5	5360 ± 30 BP	Poz-116024
151.5	11920 ± 50 BP	Poz-116025
251.5	21150 ± 120 BP	Poz-116026
351.5	29280 ± 280 BP	Poz-116027

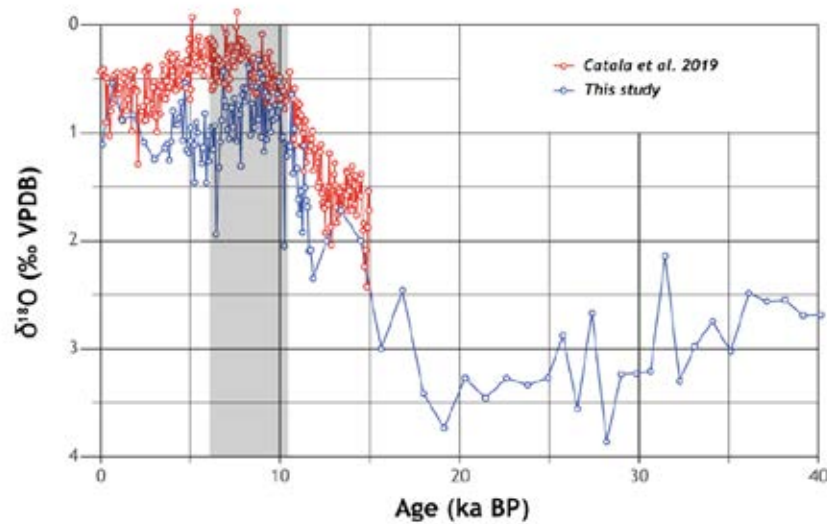


**Figure 3.** Age model based on <sup>14</sup>C-dated samples (blue marks; Table 1) for the entire length of the core showing a rather constant sedimentation rate. The section discussed in this study is marked in grey.

## RESULTS

### STABLE ISOTOPE ANALYSES

The <sup>14</sup>C based age model allows plotting the  $\delta^{18}\text{O}$  of the foraminifera and the sedimentary (trace)element contents on a common age scale and comparison to other existing records. Overall, the  $\delta^{18}\text{O}_{G.bulloides}$  record (Fig. 4) correlates well with that of a nearby core from the Alboran Sea (Català et al., 2019). Clearly visible is the change from glacial to interglacial and the short interruptions associated with the Younger Dryas and Bolling Allerød. The high-resolution age model for this part of the record also allows for a detailed comparison with records from the Eastern Mediterranean that are equally well dated and the existing alkenone-based SST reconstructions for the Alboran Sea (Martrat et al., 2004). Offsets are also observed, which are discussed later.

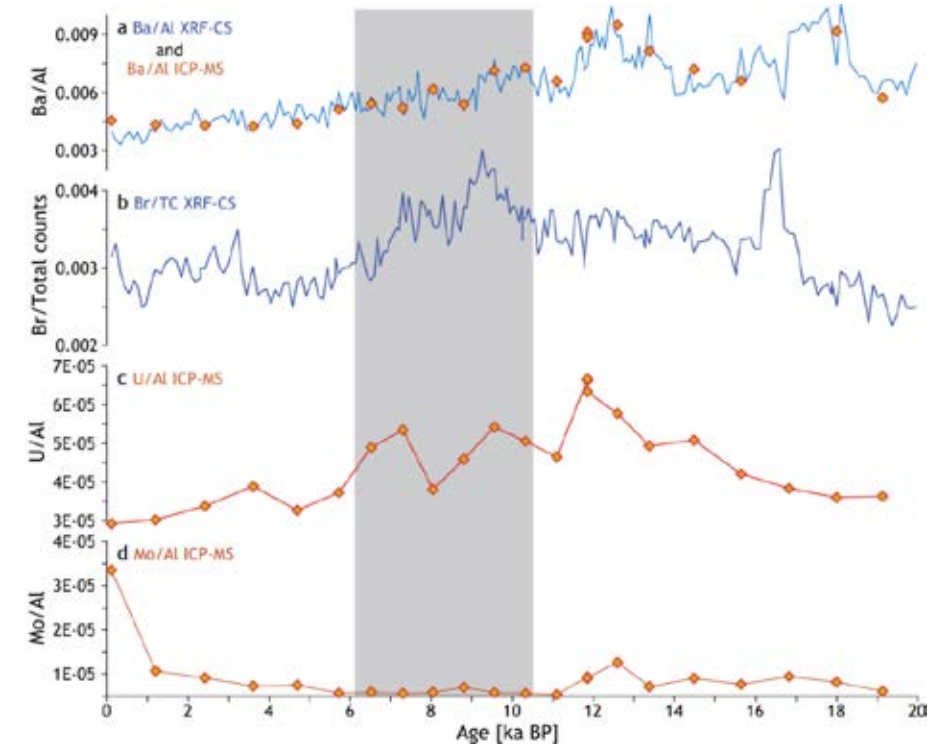


**Figure 4.** Comparison of the *G. bulloides*  $\delta^{18}\text{O}$  record of 64PE407st8 with that of Català et al. (2019). Triangles indicate the  $^{14}\text{C}$  calibration points used for the age. The interval shaded grey represents the S1-deposition interval (10.5-6.1 kyr; Grant et al., 2016).

## SEDIMENT GEOCHEMISTRY

For comparison to the Eastern Mediterranean, we mainly rely on the time of deposition of the most recent sapropel, S1. For this, we used the onset and termination of sapropel S1 as established by Grant et al. (2016).

The XRF core scanning results for Ba/Al as well as the ICP-MS based Ba/Al records (Fig. 5) show profiles typical for the area (e.g., see Martínez-Ruiz et al. (2015)), with somewhat increased productivity in the Western Mediterranean Sea, increasing well before the well-known Eastern Mediterranean sapropel S1. The ORL1 has an approximate timing of ~14.5-8.2 kyr (Rogerson et al., 2008), which fits the elevated U/Al over that entire interval (and beyond). However, Mo values clearly remain low, excluding the possibility of strong anoxic to euxinic conditions, in line with inferred dysoxic conditions in those times (Rogerson et al., 2008). The trends in Ba and Br do not match, with elevated Br/TC around 11-7 kyr and higher Ba/Al in the older part of the record (before 11 kyr). Elevated Br levels coincide with the S1-interval (Fig. 5), which also shows somewhat darker sediment (Fig. 2). Both are indicative of an elevated organic carbon content (Ziegler et al., 2008).

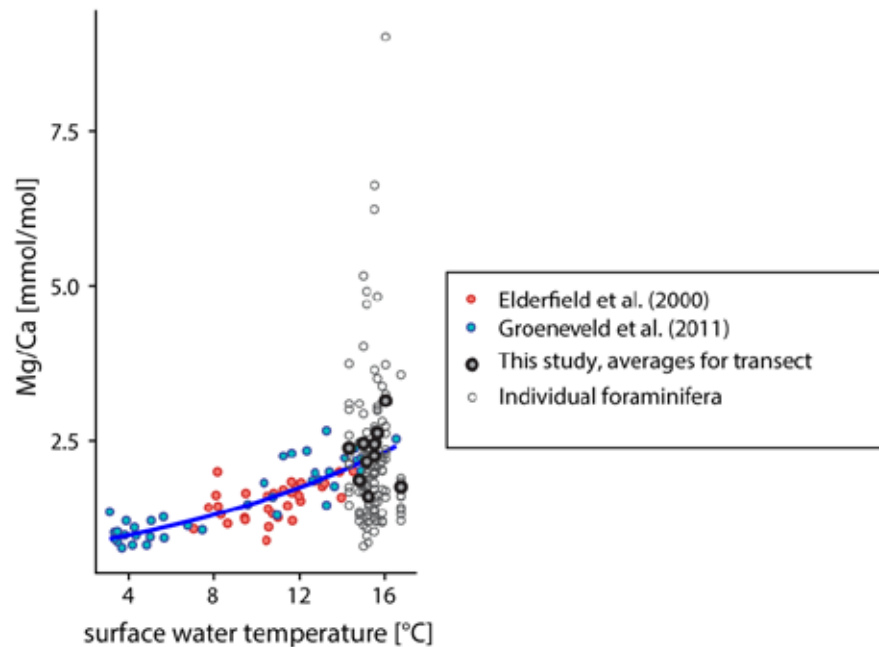


**Figure 5.** Results from the XRF core scanning and ICP-MS analyses of discrete sediment samples (indicated by the diamonds). The interval shaded grey represents the S1-deposition interval (10.5-6.1 kyr; Grant et al., 2016).

## Mg/CA-TEMPERATURE RELATIONSHIP FOR *G. INFLATA*

During collection of the plankton pump samples, weather conditions stimulated deep mixing (until at least 150 m water depth), potentially explaining the regular occurrence of *G. inflata* close to the sea surface in our samples, even though this species usually occurs 100 to 500 meters below the surface (Anand et al., 2003; Groeneveld and Chiessi, 2011). Compared to data for *G. inflata* from the North Atlantic (Elderfield and Ganssen, 2000; Groeneveld and Chiessi, 2011) the results fit well to the high temperature end of previously established calibrations (Fig. 6). From the comparison we omitted data presented by Anand et al. (2003) as they used a mild acid rinse in sample preparation, which causes these values to be somewhat offset from the data of Elderfield & Ganssen, (2000), Groeneveld & Chiessi (2011) and our data because of preferred dissolution of Mg-rich phases (Barker et al., 2003). Our single chamber LA-ICP-MS data displays

considerably more variability than the data obtained by pooling whole specimens (Fig. 6). This commonly observed larger variability could be due to several different factors, such as the larger analytical error of individual spot analyses, natural inter-specimen variability (de Nooijer et al., 2014b), lateral transport of the specimens through ocean currents (e.g., Dämmer et al., 2020; van Sebille et al., 2015), and potentially also seasonality (Wit et al., 2010) although the latter is expected to not impact deeper dwelling species such as *G. inflata* much. The few exceptionally high values for some individual spot analyses have not been omitted from the averages since they are in line with a general somewhat skewed distribution of Mg/Ca data of individual shells (e.g., Dämmer et al., 2020; Mezger et al., 2018). The relatively low values of a large number of individual measurements are in line with the specimens being brought up to the surface as a consequence of convective overturning that was observed during the cruise. But although the laser ablation derived data shows much more scatter and a slight skewing towards lower values, the trend and average Mg/Ca values align well with and further strengthen the calibration presented by Groeneveld & Chiessi (2011).

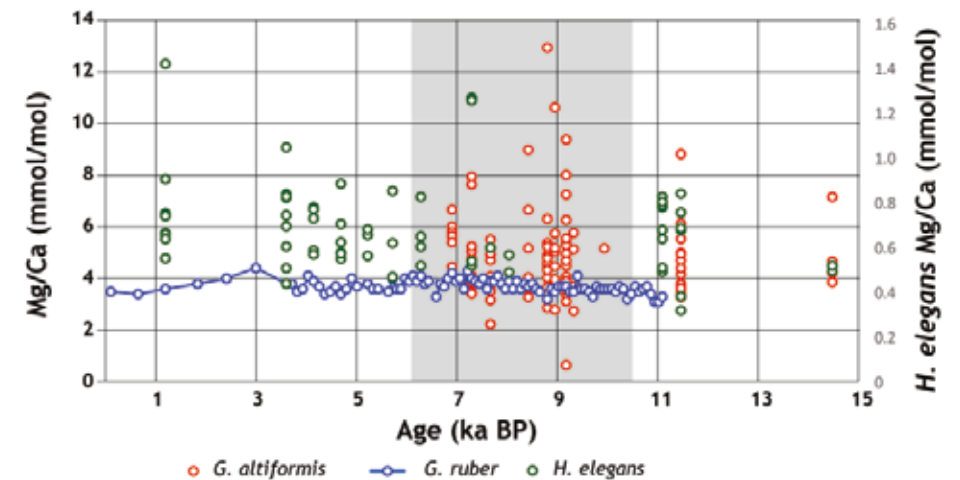


**Figure 6.** A field-based Mg/Ca-temperature calibration for *G. inflata*. The exponential regression line was based on the data from Elderfield & Ganssen (2000) and Groeneveld & Chiessi (2011). Data from this study is added to the plot to show consistency, but not used for a regression analysis as specimens were collected using plankton pump and not stratified tows.

## MG/CA AND MN/CA RECORDS FROM THE WESTERN MEDITERRANEAN SEA

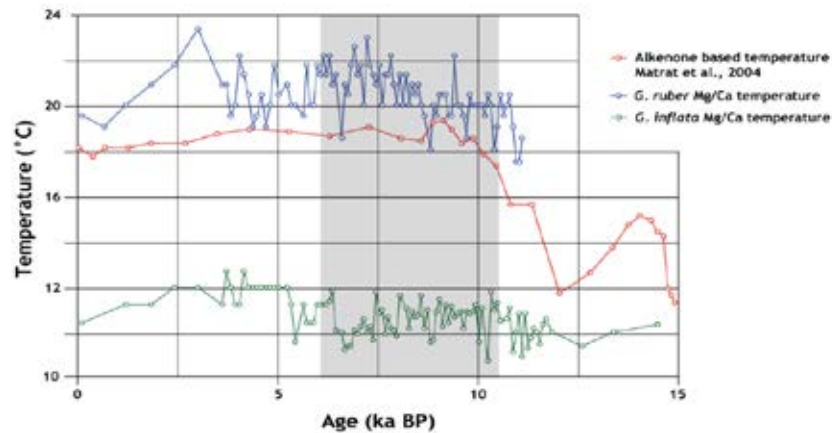
The downcore Mg/Ca shows considerable differences between species (Fig. 7). The aragonitic *H. elegans* has the lowest Mg/Ca (0-1 mmol/mol) and the *G. altiformis* highest ratios (2-12 mmol/mol). Using a range of existing calibrations for *H. elegans* (Lo Giudice Cappelli et al., 2015; Ní Fhlaithearta et al., 2010; Reichart et al., 2003) resulted in unrealistically cold but stable bottom sea water temperatures of 3-4°C throughout the entire period studied here. Temperatures could not be reconstructed from *G. altiformis* Mg/Ca values because no calibrations for this species are available to our knowledge. While absolute values might be unrealistic or cannot be obtained, since no trend or patterns can be observed in the Mg/Ca values for either species (Figs. 7 & 8), we assume relatively stable bottom water temperatures.

Using the Mg/Ca-temperature calibrations of Gray et al. (2018) for surface dwelling species *G. ruber*, Groeneveld & Chiessi (2011) for deeper dwelling species *G. inflata*, the SST were approximately 21°C and the deeper water temperatures were about 11°C during the S1-deposition interval. Both showed a slight increase of about 2°C during the early Holocene, but over slightly different timescales (Fig. 8).



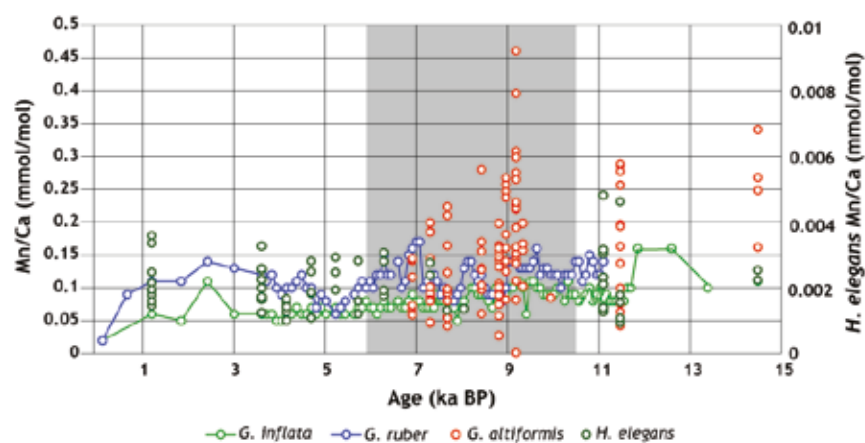
**Figure 7.** downcore Mg/Ca, for planktonic *G. ruber* (blue), and the benthic *G. altiformis* (red) and *H. elegans* (dark green). The interval shaded grey represents the S1-deposition interval (10.5-6.1 kyr; Grant et al., 2016)





**Figure 8.** Comparison between different sea water temperature proxies. The alkenone based temperature reconstruction is from the Alboran Sea (Martrat et al., 2004), the two Mg/Ca based records are from the transitional zone between the Alboran and Balearic Seas. The record for *G. ruber* starts at about 12 kyr since in samples older than that the subspecies *G. ruber ruber* used here was absent from the core. The interval shaded grey represents the S1-deposition interval (10.5–6.1 kyr; Grant et al., 2016).

Contrary to Mg/Ca, the Mn/Ca values show a decrease from the glacial to the Holocene, for all analysed species (Fig. 9). In addition to this trend, during the interval corresponding with the deposition of the sapropel S1 in the Eastern Mediterranean (10.5–6.1 ka; Grant et al., (2016)), Mn/Ca values are slightly increased, albeit that this is observed only for the benthic foraminiferal species (Fig. 9).



**Figure 9.** downcore Mn/Ca for the planktonic *G. ruber* (blue) and *G. inflata* (green), the benthic *G. altiformis* (red) and *H. elegans* (dark green). The interval shaded grey represents the S1-deposition interval (10.5–6.1 kyr; Grant et al., 2016).

## DISCUSSION

### TEMPERATURE AND SALINITY IN THE WESTERN MEDITERRANEAN SEA

Comparing the stable oxygen isotope records of piston core 64PE407st8 with that of Català et al. (2019) shows that the signals in these records are similar except for a small offset during the early Holocene (Fig. 4). This offset cannot be explained by dating (see Figs. 3 and 4). Both records are based on the same foraminiferal species and therefore, changes in e.g., seasonality and/or ecology cannot explain the observed offset, which hence must represent a true difference in the water salinity and/or temperature that these foraminifera calcified in. Català et al. (2019) showed a similar offset in the isotopic signals between *G. bulloides*-based stable oxygen isotopic records from the Alboran Sea and a record from the Minorca Drift. Whereas in that study the offset is similar throughout the record, here we observe such an offset only during the time interval corresponding to the deposition of sapropel S1 in the Eastern Mediterranean, approximately from 10 ka BP to 5 ka BP (Fig. 4). During that time interval the oxygen isotopic composition at what is roughly the interface between the Balearic and Alboran seas is more in line with that observed at the Minorca drift. This indicates that at that time, either the temperature and/or the sea water stable isotopic composition and hence salinity was deviating more from those in the Atlantic Ocean, which may be indicative of reduced exchange between the Mediterranean and the Atlantic. The fact that such an offset was not observed in earlier studies in the Alboran and Balearic Seas might be due to the specific location of the core studied here, at the interface between the well-mixed Alboran Sea and the Balearic, which has a true Mediterranean signature.

The down core changes in Mg/Ca and reconstructed sea surface temperatures shows overall similar temperatures as today. This is in line with earlier studies using the alkenone unsaturation index ( $U^{K'_{37}}$ ) (Cacho et al., 1999; Martrat et al., 2004; Morcillo-Montalbá et al., 2021) and this is also expected based on the  $\delta^{18}O$  data presented here. This confirms that the cleaning protocol used here was appropriate and that our analyses are robust. The results also reflect the different depths recorded by *G. ruber*, *G. inflata* and the benthic species considered here. The temperature gradient of approximately 10 °C from the surface water (*G. ruber* about 21 °C) to the deeper water (*G. inflata*, about 11 °C) remains relatively stable within error over the entire interval studied, though the absolute values for both surface and deeper water increase during the Holocene (Fig. 8).

## CHANGES IN (DE)OXYGENATION, PRODUCTIVITY, AND CIRCULATION IN THE WESTERN MEDITERRANEAN

The Mn/Ca values for *G. ruber* are too high to represent ratios recorded at the time the organism was living, with values at about 150  $\mu\text{mol/mol}$  (Fig. 9). Controlled growth (Barras et al., 2018; Munsel et al., 2010) and environmental calibrations (Steinhardt et al., 2014) have shown that these values for *G. ruber* are unrealistic. The core top sample however shows a value close to what would be expected for *G. ruber*, suggesting a gradual alteration of the original *G. ruber* Mn/Ca values deeper in the core. The Mn/Ca values for *G. inflata* are more in line with what would be expected at depth in the Mediterranean, at relatively high but more or less constant ( $\pm 100 \mu\text{M}$ ) Mn/Ca values. These values are similar to those measured for *G. scitula* in the southern Indian Ocean (Davis et al., 2020; Steinhardt et al., 2014). Similarly high values were reported before, also for shallow living foraminifera, in upwelling areas around Antarctica (Marr et al., 2013).

For reconstructing bottom water changes in Mn/Ca we used both *H. elegans* and *Gyroidina altiformis* to make a composite Mn/Ca record. It is very unlikely that both species would have a similar response to changes in bottom water Mn/Ca (Barras et al., 2018; Koho et al., 2015). *G. altiformis* is a shallow infaunal species (Fontanier et al., 2002) while *H. elegans* is epifaunal (Murray, 2013). Still, the Mn/Ca values of both species suggests that during sapropel times Mn/Ca<sub>sw</sub> was slightly higher in the bottom water. Today, the Mediterranean has a typical open ocean concentration profile, with elevated concentrations at the sea surface decreasing to low and relatively uniform concentrations at depth (Middag et al., 2022). At the sea surface Mn is supplied by dust, which is subsequently scavenged and transported to the sea floor. Whereas enhanced dust inputs would increase both surface and deep-water Mn, during sapropel times we mainly observe an increase in the bottom water. As during sapropel time it is suggested that higher riverine inputs from the river Nile and the North African river system might have supplied additional freshwater to the basin. The inputs related to fresh water are, however, relatively minor for the Mediterranean (Elbaz-Poulichet et al., 2001) and should affect both surface and deep-water. The most likely source for Mn-rich deep-water in our record is the Eastern Mediterranean Sea which during sapropel times was characterized by strongly reducing conditions, resulting in the massive release of Mn from the sediments (De Lange et al., 2008). This Mn must have been transported to the western basin and resulted in higher bottom water Mn and hence benthic foraminiferal Mn/Ca. Although the western Mediterranean remained fully oxygenated, during sapropel times transport of Mn across the entire western basin was possible due to the relatively

slow reaction kinetics of dissolved Mn in sea water. Similarly, zones of elevated Mn are observed in the Pacific and Indian Ocean after the Mn being leached from the continental margins underlying the locally very intense oxygen minimum zones (Mangini and Eisenhauer, 1990; Saager et al., 1989). In the Arabian Sea it has been shown that at times of an intensified oxygen minimum zone Mn transport across the basin is also enhanced (Schenau et al., 2002) similar to what is observed here.

The enhanced bottom water Mn during sapropel times could, alternatively, be due to enhanced productivity and local areas with reducing sedimentary conditions in the Western Mediterranean. However, there is no evidence for the existence of these areas (Fig. 5). Moreover, when local productivity would have resulted in enhanced local regeneration of Mn this should have resulted in a steady additional supply rate of Mn. In contrast, the record of *G. altiformis* is highly skewed with highest Mn concentrations at the onset of sapropel formation in the eastern basin, followed by a gradual decrease and overall low values after the sapropel. Such an initial rapid increase in Mn points to the pulsed input of Mn related to the sudden shift of the Eastern Basin into anoxia. After the Mn has been leached from the sediment, it is transported away, after which in a new steady state the fluxes will gradually return to pre-sapropel times.

The observation of elevated Mn concentrations at mid-depth in the westernmost part of the Western Mediterranean shows that the flow of water from the eastern basin towards the west continued, also during sapropel times, in line with previous studies (e.g., Rohling & De Rijk, 1999). This implies that the current anti-estuarine flow continued also at sapropel times and connected the eastern and western basins. If the western basin had developed a separate anti-estuarine circulation loop this would not have resulted in extra Mn in the deeper western part. Still, it is also clear that this flow must have been very slow and local ventilation, potentially in the Gulf of Lion, likely supplied additional oxygen to the locally well-ventilated deep sea.

A continued, albeit weakened, anti-estuarine circulation in the whole Mediterranean Sea has several important implications for the reconstruction of the conditions that resulted in sapropel formation. First, productivity in the Eastern Mediterranean must have been higher than what would have been necessary with circulation shut down. Currently it is, however, not possible to quantify the relative importance of this factor as a slower circulation, or circulation with large temporal changes would have re-

sulted in very similar records. However, considering elemental budgets they differ very much and work on older sapropels already suggested that some import of redox sensitive metals must have continued during sapropel times (Nijenhuis et al., 1999). Second, deep water formation must, to a certain extent, have continued. One of the areas that may have played a role in the formation of small pulses of deeper water during sapropel times is the Aegean Sea. Local cooling in this shelf sea might have repeatedly resulted in the formation of denser water spilling into the Eastern Mediterranean (Ní Fhlaithearta et al., 2010). Although driving a continued overall anti-estuarine circulation, these pulses were apparently never large enough to substantially ventilate the local deeper part of the basin, except for the Sapropel S1 interruption that coincides with the so-called '8.2 kyr event' (Rohling and Pälike, 2005). At that time the anti-estuarine circulation was temporarily strong enough to interrupt sapropel formation, although this is not visible in the record presented here. Potentially such an invigorated circulation could have transported extra Mn to the western basin. However, the balance between circulation, Mn scavenging and final transport across the basin is difficult to predict. Lastly, our results indicate that during the start of S1, when anoxia was strongest in the Eastern Mediterranean basin, this may have reinforced the dysoxic conditions observed in the western basin. Our results indicated that the water mass from the Eastern Mediterranean and its biogeochemical properties must have reached far into the western basin at those times.

## CONCLUSIONS

We here show that anti-estuarine circulation continued through sapropel formation, to a certain extent maintaining a link between the Western and Eastern Mediterranean basins. Although the basins had a strongly contrasting biogeochemistry at that time, Mn was likely transported across the entire basin. The amount of Mn deposited in the western basin is limited when compared with the removal from the eastern basin, suggesting that either slow kinetics allowed effective removal from the Mediterranean, or there is a yet undiscovered zone with highly elevated Mn concentrations between the Eastern and Western Mediterranean basins.

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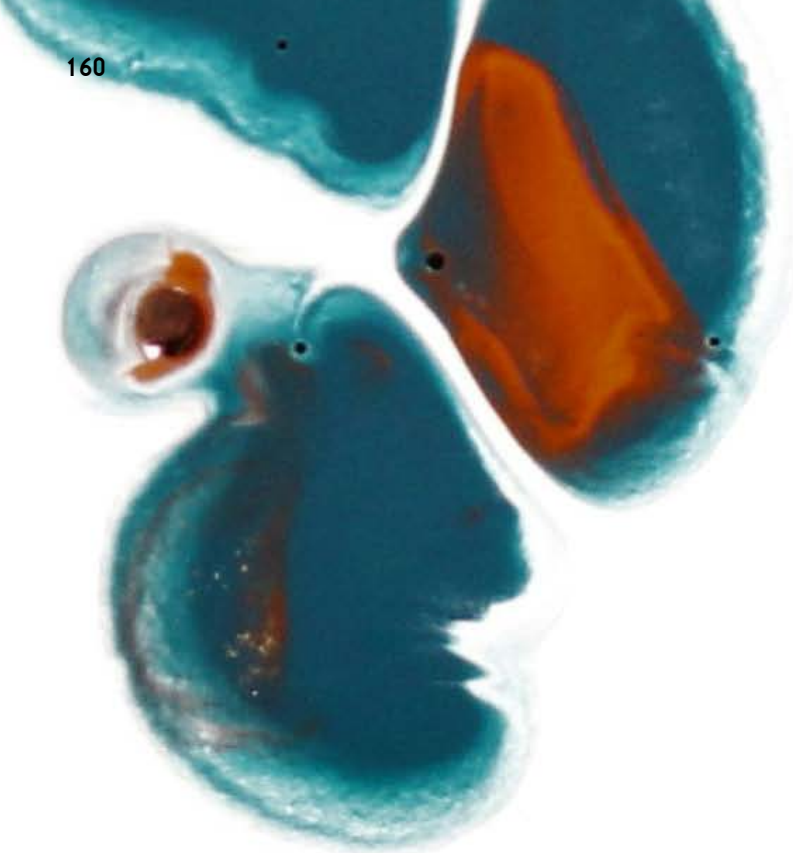
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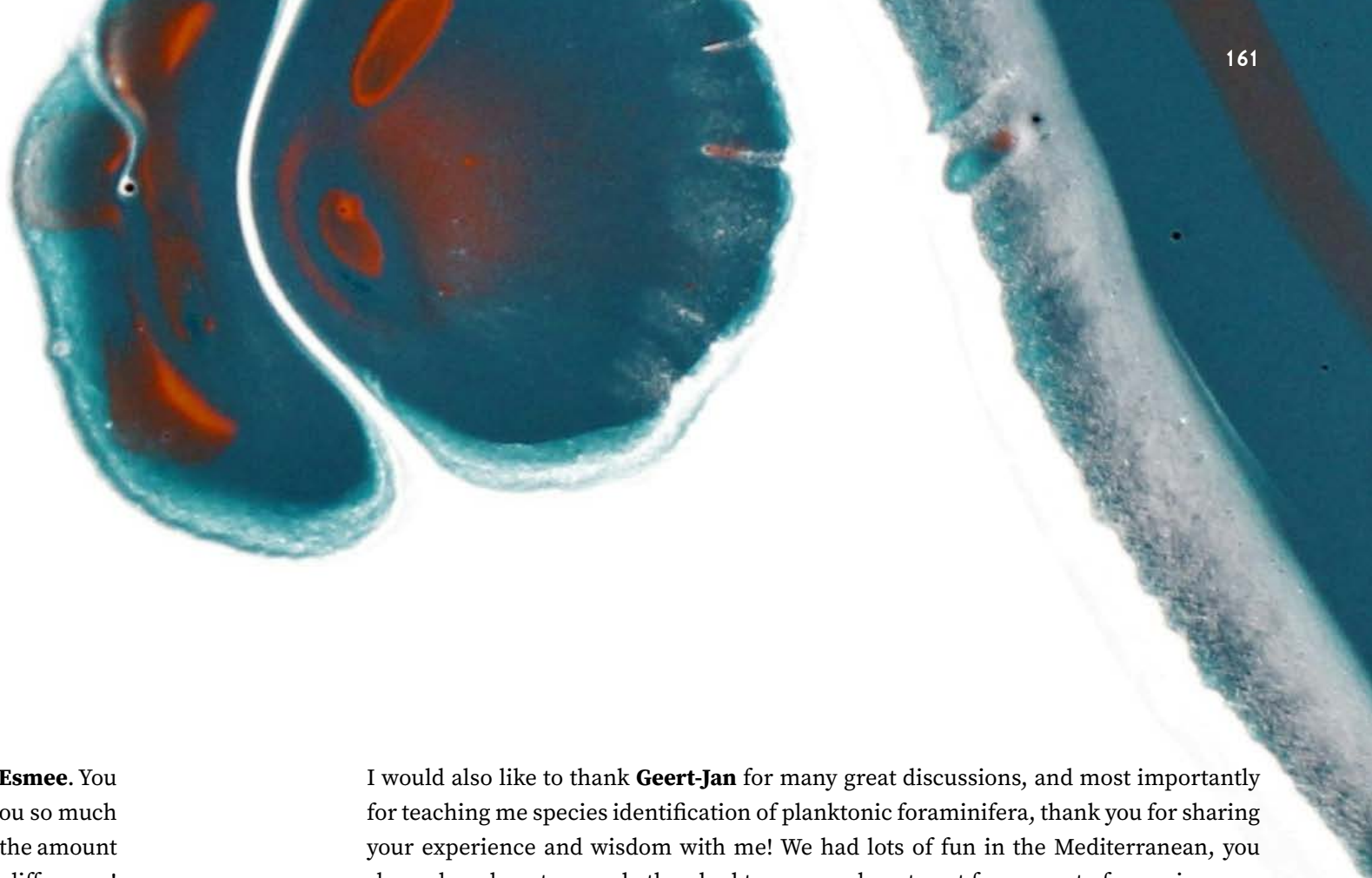




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# ABOUT THE AUTHOR



Linda Karoline Dämmer was born on 2<sup>nd</sup> of June, 1990, in Wuppertal, Germany.

After finishing school, Linda signed up for a Bachelor of Science in Geosciences at University of Bonn, where she focussed especially on palaeontology and geochemistry. For the undergraduate thesis Linda worked on eclogites from southern Bulgaria, a project that not only involved exciting field work but also lots of interesting work in the laboratories of the university, a balance that was always important for Linda. After finishing her Bachelor's degree in 2014, Linda enrolled at Trinity College Dublin's Master of Science in Environmental Sciences programme to deepen her understanding of environmental chemistry. Her Master thesis then again involved her favourite subjects: geochemistry and palaeontology. Linda analysed rare earth elements in the skeletons of cold-water corals from different locations in the northeast Atlantic Ocean from different times in the Holocene, and looked at how they correlate with the chemical fingerprints of the corresponding ocean currents to see if they could be used as proxies for ocean current pattern changes. The excitement for marine carbonates and proxy work then lead Linda to start a PhD on a very similar topic at the Royal Netherlands Institute of Sea Research in early 2016. During her PhD Linda tried to improve our understanding of biomineralization processes in foraminifera, especially on aspects of magnesium incorporation, a topic crucial for our paleoclimate reconstructions. Only if we know how and why trace elements get incorporated in which ratios into marine biogenic carbonates can we use them confidently to reconstruct paleoenvironmental parameters.

In addition to her PhD work on foraminiferal biomineralization, Linda was also involved in side projects and cooperations with other researchers where biomineralization processes in other marine calcifiers were studied, for example the impact of ocean acidification on pteropoda.

During her PhD Linda also got involved with several science communication projects and enjoys stimulating the curiosity for science, especially paleoclimatology, in others.

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