

# Synchrotron X-ray microscopy of marine calcifiers: how plankton record past climate change

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**Abstract.** We have used STXM and PEEM to reveal the underpinning chemistry and nanoscale structure behind palaeo-climate geochemical signatures, such as trace Mg in shells- proposed proxies for palaeo-ocean temperature. This has allowed us to test the chemical assumptions and mechanisms underpinning the use of such empirical proxies. We have determined the control on driving chemical variations in biogenic carbonates using STXM at the absorption edge of Mg, B, and Na in the shells of modern plankton. The power of these observations lies in their ability to link changes in chemistry, microstructure, and growth process in biogenic carbonate to environmental influences. We have seen that such changes occur at length scales of tens of nanometres and demonstrated that STXM provides an invaluable route to understanding chemical environment and key heterogeneity at the appropriate length scale. This new understanding provides new routes for future measurements of past climate variation in the sea floor fossil record.

## 1. Introduction

Earth's atmospheric CO<sub>2</sub> is now at a higher level than it has been at any time in the last 15 million years<sup>1,2</sup>. This is despite the fact that about one third of atmospheric anthropogenic CO<sub>2</sub> has been absorbed by the oceans, moderating climate change. Consequent decrease in seawater carbonate ion concentration may affect the ability of marine organisms to build their calcium carbonate. Past climate change, recorded in shells recovered from ocean sediment, give insights into current variation, but the methods of decoding past climate from calcified organisms remain largely empirical. One such class of organisms are the single-celled foraminifera, that live in the water column as planktonic species or at in or near the sediment as benthic species. Because they are ubiquitous and readily identified,



studies of foraminiferal microfossils have formed the backbone of a huge body of work devoted to understanding past marine environments over Earth history.

There is abundant evidence in the geological record, from carbonate minerals that form the hard parts of marine organisms such as plankton (recovered from ocean floor sediment in drilled cores that represent a record back in time), that  $\text{CaCO}_3$  mineral polymorphs making up those marine calcifiers have been influenced by the ratio of Mg to Ca concentrations in ancient oceanic waters, stretching back tens of millions of years<sup>3</sup>. The incorporation of Mg into  $\text{CaCO}_3$  hard parts of marine calcifiers, as well as its incorporation into abiogenically-precipitated carbonates, is dependent on temperature, with Mg solid solubility in  $\text{CaCO}_3$  increasing with increasing temperature for simple thermodynamic reasons. Although Mg/Ca ratios in biogenic and abiogenic carbonates are not identical, when grown in seawater of identical composition, the Mg/Ca ratio of foraminiferal calcite is a widely accepted and applied empirical “proxy” for ocean temperature<sup>4,5</sup>. The construction of Mg/Ca records from foraminifera preserved in ocean sediments has been instrumental in developing our understanding of past global climate, but the mechanisms behind this proxy have remained largely unknown. Similar proxies based on other elements have also been proposed in recent years, including the use of boron in carbonates as an indicator of variations in ocean pH, of sodium as an indicator of salinity, and of iodine as an indicator of oxygen content. In each case the application is empirical, based on observed correlations between external drivers and internal chemistry. We have applied X-ray microscopy techniques to the problems of biomineral trace element incorporation in foraminifera to reveal, for the first time, the modes of incorporation and to provide a basis of understanding of how geochemical palaeoproxies operate.

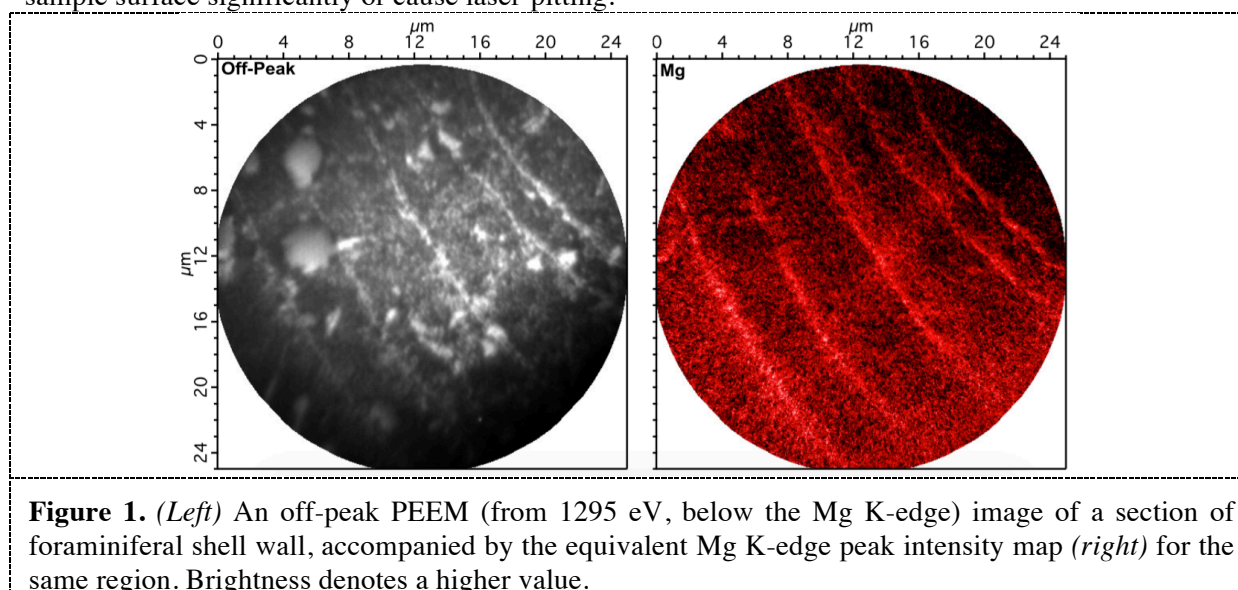
## 2. Synchrotron X-ray microscopy of foraminifera

Spectroscopic techniques to investigate element-specific atomic coordination within a structure have been developed over the last few decades at synchrotron light sources. Various forms of X-ray absorption spectroscopy (XAS), including Near Edge X-ray Absorption Fine Structure (NEXAFS/XANES) and Extended X-ray Absorption Fine Structure (EXAFS) spectroscopy, have been applied to investigate element coordination in bulk biomineral samples<sup>6-9</sup>. The development of synchrotron X-ray microscope instrumentation, providing nano-scale monochromatic X-ray probes, has added spatial discretion to these techniques, allowing the simultaneous nanoscale mapping and spectroscopy of specific elements within a structure<sup>10-13</sup>. Techniques such as photo-electron electron microscopy (PEEM) and scanning transmission X-ray microscopy (STXM) provide a route to developing a comprehensive picture of trace element incorporation in biominerals such as foraminifera.

### 2.1. PEEM studies of Mg in foraminifera

We have investigated the distribution of Mg, Na, S and Fe in foraminifera, brachiopod and ostracod specimens using the Spin-resolved Photo-Emission Electron Microscope (SPEEM) branch of the BESSY II synchrotron beamline UE49-SPEEM, Berlin. Two forms of data were collected during analysis: spectral image stacks (Mono S scan) and difference maps between specified on- and off-peak energies (X scan). Data were collected at either the K or L2-3 edges of Mg, Na, S and Fe. Here we focus on the results for Mg. A number of challenges arose in applying this technique to resin-mounted calcium carbonate specimens. The specimens considered here are electrical insulators, which poses a significant surface charging problem and consequent image distortion. The effect of ionising radiation on the specimens is largely unknown, but the samples showed no observable changes during the experiment, suggesting that there were no significant effects due to potential radiation damage imparted on the samples. The PEEM technique is extremely surface-sensitive (top 10s of nm), making the quality of the results particularly sensitive to the condition of the sample surface, and the sample preparation techniques.

Despite these difficulties PEEM images were obtained which revealed the internal heterogeneity of multiple trace elements in foraminifera, which tends to follow systematic banded patterns, normal to the growth axis of the shell<sup>10,14-16</sup>. Elemental maps at the Mg K-edge (Figure 1) reveal a variation in the intensity, concomitant with the bright bands seen in the off-peak image, suggesting that these bands contain elevated Mg, in agreement with previous studies<sup>10,14,15</sup>. The corroboration of the results with those of past studies demonstrates the ability of the PEEM instrument to map trace elements in carbonate minerals. Furthermore, the identification of Mg banding in the structure, and the shape of the Mg NEXAFS spectra identified with the PEEM instrument are in agreement with a more extensive analyses of foraminiferal Mg, using an alternative X-ray spectroscopy technique, STXM<sup>10</sup>. The nano-scale resolution of the PEEM instrument offers a significant advance from most previous methods, and compared to the commonly-used laser ablation mass spectrometry techniques PEEM does not alter the sample surface significantly or cause laser-pitting.



## 2.2. Scanning Transmission X-ray Microscopy (STXM) studies of Mg and B in foraminifera

We have characterised the coordination of Mg, B and Na in two disparate species of symbiont-bearing foraminifera using Near-Edge X-ray Absorption Fine Structure (NEXAFS) spectroscopy. Both the benthic *Amphistegina lessonii* and the planktic *Orbulina universa* exhibit Mg-banding<sup>15,17-19</sup>, which we have confirmed by electron microprobe. Data were gathered at the STXM branch of beamline 11.0.2 at the Advanced Light Source (ALS, Berkeley, CA). Raster X-ray absorption images map Mg, B and Na distributions at much higher resolution than the electron microprobe (Figure 2). This high-resolution mapping reveals that the broad (~2–3 μm) bands observed in *A. lessonii* by the electron probe were in fact made up of sequential finer (<500 nm) bands in close proximity, revealing that banding is much finer-grained than previously thought. NEXAFS spectra gathered at the Mg-edge from on- and off-band regions in *A. lessonii* and *O. universa* were identical within instrumental error, as is evident from the featureless (to within instrumental noise) residuals in Figure 3.

The Mg banding could either be produced by variations in the concentration of Mg hosted in a single phase, or be the result of a multi-host-phase, where background Mg environment is augmented by an alternative phase in the bands. Our NEXAFS spectra demonstrate the sensitivity of this method to changes in the chemical environment of the Mg ion: closely related mineral phases produce disparate spectra (Figure 3). Therefore, we would expect a single-host-phase system (Mg in a single homogeneous chemical environment) to produce uniform spectra throughout the test and the spectra of

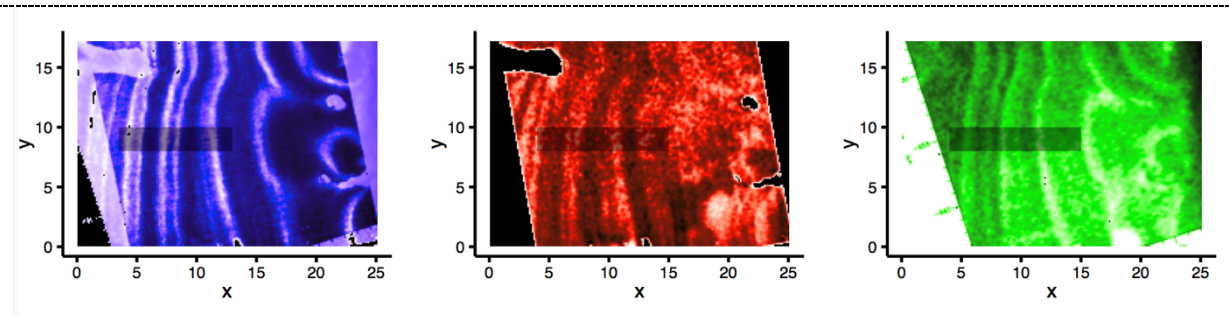
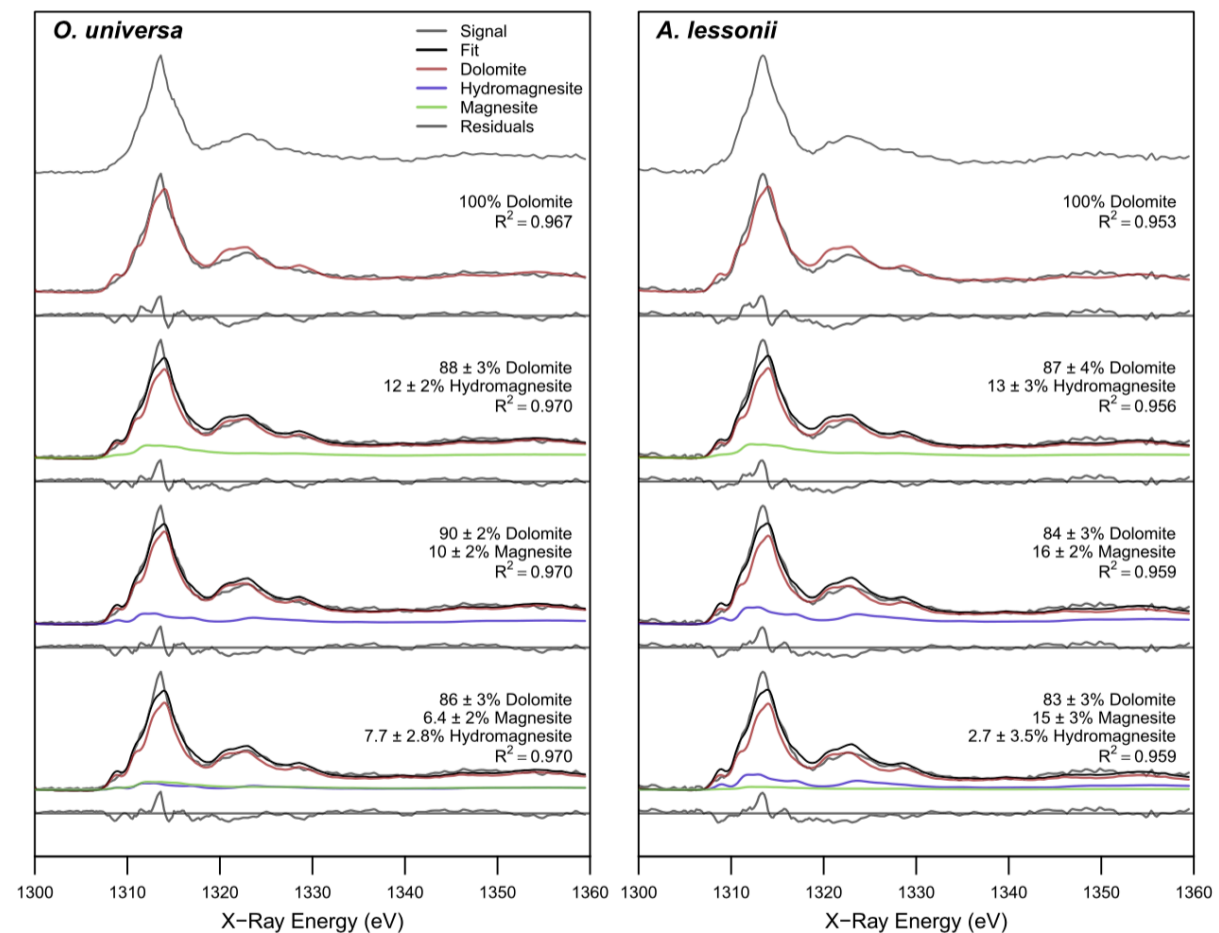
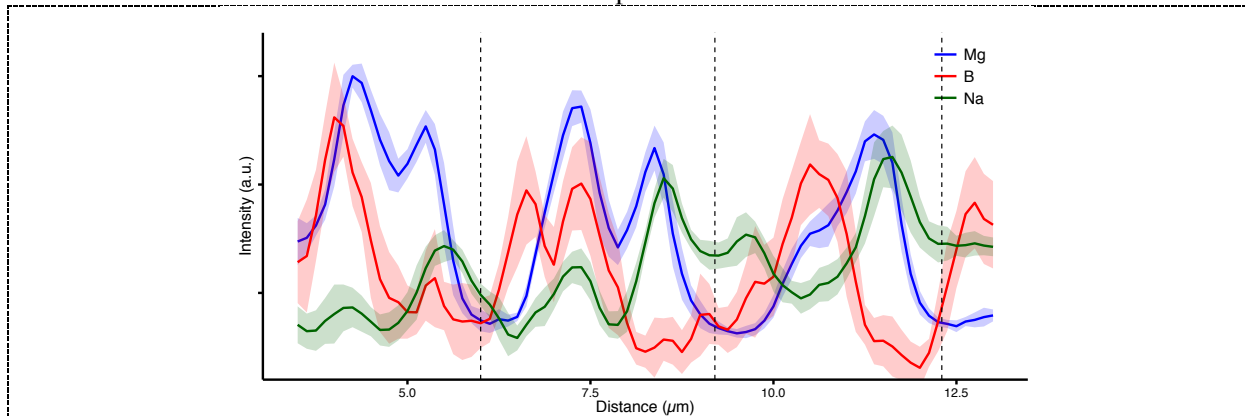


Figure 2. Mg (left), B (centre) and Na (right) distributions measured by STXM across the shell wall of *Orbulina universa*. Axes scales are labelled in  $\mu\text{m}$ . The heterogeneous banding is evident perpendicular to the growth direction for each element, although maxima in each elemental concentration do not coincide in position and banding is offset/out of phase between elements. The grey bar corresponds to the composition profiles show in Figure 4 below.

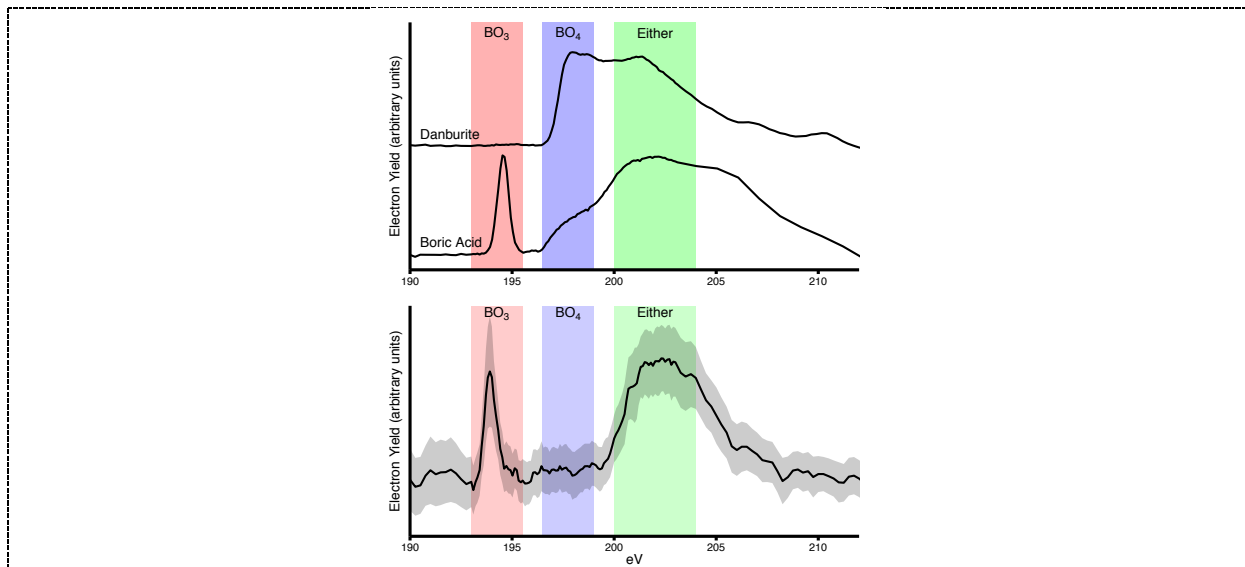


**Figure 3.** NEXAFS spectra from image stacks at the Mg-edge in high-Mg areas from *O. universa* and *A. lessonii*, compared to the candidate minerals dolomite, hydromagnesite and magnesite. Spectra from low-Mg areas are identical. Each fit consists of one or more candidate spectra combined with a linear background. The dolomite spectrum alone is a good fit for the foraminiferal Mg. The fit is marginally improved by adding extra phases, but the shape of the residuals (shown below each model) does not alter significantly, and the increase in goodness of fit (indicated by  $R^2$ ) is insignificant.

a multi-host-phase system (with multiple Mg chemical environments) to vary between the on- and off-band regions. The presence of B and Na banding in the shell demonstrates a general chemical variation associated with such bands, which have previously been identified with daily calcification events (Figure 4). The boron NEXAFS spectra (Figure 5) measured from both high-boron and low-boron areas suggest that the boron is incorporated into the structure as  $\text{BO}_3$ , replacing the carbonate group in the rhombohedral calcite<sup>16</sup>. The sodium spectra are more equivocal and do not allow us to infer the chemical environment of Na in these samples.



**Figure 4.** Relative intensities (in arbitrary units, but related to concentrations) of Mg, B and Na across the shell wall of *O. universa*, taken from the grey shaded areas indicated in Figure 2. The cyclicity of compositional banding is similar for each element, but detailed comparison demonstrates that concentrations peak at different stages in each shell growth cycle.



**Figure 5.** NEXAFS spectra at the B-edge for *O. universa* (below) compared to danburite (tetrahedral B) and boric acid (trigonal B) standards (above). The peak at 190 eV in the *O. universa* sample corresponds to  $\text{BO}_3$  within the sample, and there is no evidence for the presence of  $\text{BO}_4$ , suggesting that borate substitutes directly for carbonate in the calcite shell.

### 3. Discussion and conclusions

Our results provide a fundamental step forwards towards a mechanistic understanding of the Mg/Ca palaeotemperature proxy and the boron proxy for pH, as well as for the general mechanisms of

biomineralisation. By conclusively demonstrating the ideal substitution of Mg into the calcite lattice we have confirmed a long-held, yet untested assumption behind the Mg/Ca proxy, supporting the presence of an inorganic thermodynamic connection between sea- water temperature and foraminiferal Mg/Ca. We demonstrate that foraminiferal Mg is not hosted in organic molecules, or in a secondary interstitial mineral or organic environment. Furthermore, the consistency of the Mg coordination throughout the shell indicates that foraminifera employ a uniform mechanism of crystal growth. This supports the links between calcite-bound trace elements (like Mg) and the external environment upon which palaeo-proxies rely. Our work on boron demonstrates that it substitutes for  $\text{BO}_3$  in the shell and is similarly heterogeneous, showing banding at a sub- $\mu\text{m}$  length scale. The B proxies ( $\delta^{11}\text{B}$  and B/Ca) have the potential to provide a record of the entire ocean carbonate system throughout Earth history. However, these proxies require the sole incorporation of tetrahedral  $\text{B}(\text{OH})_4^-$  from seawater, which has thus far been missing in the literature. Our coordination data uphold the underlying theory behind the B palaeoproxies, and give a new confidence to the interpretation of B records of past ocean carbonate chemistry. This helps validate their use as a tool to understand ocean carbonate chemistry, and the effects of ocean acidification.

We have determined the control on driving chemical variations in biogenic carbonates using STXM at the absorption edge of Mg, B, and Na in the shells of modern plankton. The power of these observations lies in their ability to link changes in chemistry, microstructure, and growth process in biogenic carbonate to environmental influences. We have seen that such changes occur at length scales of tens of nanometres and demonstrated that STXM provides an invaluable route to understanding chemical environment and key heterogeneity at the appropriate length scale. This new understanding provides new routes for future measurements of past climate variation in the sea floor fossil record.

### Acknowledgements

We thank David Nicol, Iris Buisman and Martin Walker for invaluable technical assistance, and James Bryson for his help with synchrotron data collection. Jean DeMouthe (California Academy of Sciences) and Mike Rumsey (Natural History Museum, London) provided of B-containing minerals for use as references. This work was funded by NERC, Jesus College (Cambridge) and the US Department of Energy (via ALS)

### References

- [1] M Pagani, J C Zachos, K H Freeman, B Tipple, and S Bohaty (2005) Marked decline in atmospheric carbon dioxide concentrations during the Paleogene. *Science*, 309: 600–603.
- [2] A K Tripathi, C D Roberts, and R A Eagle (2009) Coupling of  $\text{CO}_2$  and ice sheet stability over major climate transitions of the last 20 million years. *Science*, 326: 1394–1397.
- [3] J B Ries (2010) Review: geological and experimental evidence for secular variation in seawater Mg/Ca (calcite-aragonite seas) and its effects on marine biological calcification. *Biogeosciences*, 7: 2795–2849.
- [4] D W Hastings, A D Russell and S R Emerson (1998) Foraminiferal magnesium in *Globeriginoides sacculifer* as a paleotemperature proxy. *Paleoceanography*, 13: 161–169.
- [5] H Elderfield and G Ganssen (2000) Reconstruction of temperature and  $\delta^{18}\text{O}$  of surface ocean waters using Mg/Ca of planktonic foraminiferal calcite. *Nature*, 405: 442–445.
- [6] A A Finch and N Allison (2007) Coordination of Sr and Mg in calcite and aragonite. *Mineralogical Magazine*, 71:539–552, 2007.
- [7] A A Finch and N Allison (2008) Mg structural state in coral aragonite and implications for the paleoenvironmental proxy. *Geophysical Research Letters*, 35: L08704.
- [8] L C Foster, A A Finch, N Allison, C Andersson, and L J Clarke (2008) Mg in aragonitic bivalve

- shells: Seasonal variations and mode of incorporation in *Arctica islandica*. *Chemical Geology* 254: 113–119.
- [9] L C Foster, N Allison, A A Finch, and C Andersson (2009) Strontium distribution in the shell of the aragonite bivalve *Arctica islandica*. *Geochem Geophys Geosyst*, 10, Q03003.
- [10] O Branson, S A T Redfern, T Tyliszczak, A Y Sadekov, G Langer, K Kimoto and H Elderfield (2013) The coordination of Mg in foraminiferal calcite. *Earth and Planetary Science Letters*, 383: 134–141.
- [11] J-P Cuif, Y Dauphin, G Nehrke, J Nouet, and A Perez-Huerta (2012) Layered growth and crystallization in calcareous biominerals: Impact of structural and chemical evidence on two major concepts in invertebrate biomineralization studies. *Minerals*, 2: 11–39.
- [12] M Cusack and A Freer (2008) Biomineralization: Elemental and organic influence in carbonate systems. *Chem Rev*, 108:4433–4454.
- [13] F Farges, A Meibom, A M Flank, P Lagarde, M Janousch, and J Stolarski (2009) Speciation of Mg in biogenic calcium carbonates. *Journal of Physics: Conference Series*, 190
- [14] S M Eggins, P de Deckker, and J Marshall (2003) Mg/Ca variation in planktonic foraminifera tests: implications for reconstructing palaeo-seawater temperature and habitat migration. *Earth and Planetary Science Letters*, 212: 291–306.
- [15] S M Eggins, A Sadekov, and P de Deckker (2004) Modulation and daily banding of Mg/Ca in *Orbulina universa* tests by symbiont photosynthesis and respiration: a complication for seawater thermometry? *Earth and Planetary Science Letters*, 225: 411–419.
- [16] O Branson, K Kaczmarek, S A T Redfern, S Misra, G Langer, T Tyliszczak, J Bijma and H Elderfield (2015) The coordination and distribution of B in foraminiferal calcite. *Earth and Planetary Sciences Letters*, 416: 67-72.
- [17] J Erez, J (2003) The source of ions for biomineralization in foraminifera and their implications for paleoceanographic proxies. *Reviews in Mineralogy and Geochemistry*, 54: 115–149.
- [18] D Kunioka, K Shirai, N Takahata, Y Sano, T Toyofuku and Y Ujiie (2006) Microdistribution of Mg/Ca, Sr/Ca, and Ba/Ca ratios in *Pulleniatina obliquiloculata* test by using a NanoSIMS: Implication for the vital effect mechanism. *Geochem. Geophys. Geosyst.* 7: Q12P20.
- [19] A Y Sadekov, S M Eggins, P de Deckker (2005) Characterization of Mg/Ca distributions in planktonic foraminifera species by electron microprobe mapping. *Geochem. Geophys. Geosyst.* 6: Q12P06.