Modulation and daily banding of Mg/Ca in *Orbulina universa* tests by symbiont photosynthesis and respiration: a complication for seawater thermometry?

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Abstract

The Mg/Ca composition of calcium carbonate tests (shells) secreted by planktonic foraminifera is increasingly being employed to estimate past seawater temperatures and reconstruct paleocean and climate records spanning hundreds of thousands of years. We show, using two high-resolution microanalysis techniques, that the final chamber of the planktonic foraminifera *Orbulina universa* typically comprises between three and six paired, low and high Mg, growth bands. The number and spacing of these bands is consistent with a diurnal origin, modulated by changing pH within the foraminiferal microenvironment due to the day–night, photosynthesis–respiration cycle of algal symbionts. The amplitude of Mg/Ca variation within individual tests and across many daily growth bands cannot be accounted for by seawater temperature in the shallow, euphotic zone habitat of *O. universa*. Our results indicate the Mg/Ca composition of calcite precipitated by *O. universa* in nature is strongly influenced by diurnal changes in the biological activity of algal symbionts and the host foraminifer. This brings into question the fundamental premise often made in applying Mg/Ca palaeoseawater thermometry, that the Mg/Ca composition of foraminiferal calcite is determined by seawater temperature, and whether the Mg/Ca composition of other planktonic species that are more widely used for palaeoseawater thermometry are subject to similar influences.

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1. Introduction

The recent development and application of foraminiferal Mg/Ca seawater thermometry to palaeocean-
nography has been driven by the sensitivity of bulk test Mg/Ca compositions to seawater temperature (Mg/Ca increases by $9\pm 1\% \, ^\circ C^{-1}$) [1–4], the high precision with which Mg/Ca can be measured on bulk samples that comprise 5 to 50 fossil tests [5], and the abundance of fossil foraminifera in seafloor sediments from which continuous records of seawater temperature spanning hundreds of thousands of years can be assembled (e.g., [2,6–8]). The only significant problem attributed to Mg/Ca seawater thermometry, beyond the need to carefully clean tests to remove Mg-rich contaminants, is the partial dissolution of more Mg-rich fossil tests with increasing depth in the oceans [2,9–12]. If not corrected for, this lowers bulk test Mg/Ca compositions and results in underestimation of past seawater temperatures [9,11,12]. Nonetheless, some laboratory culture studies have suggested that test Mg/Ca composition might also be influenced by factors other than temperature, in particular, by changes in seawater pH or carbonate ion composition [13,14]. Until now, these effects have not been demonstrated outside the laboratory nor have they been considered significant in nature.

For this study, we chose to investigate the distribution of Mg/Ca within subfossil tests of the planktonic foraminifera *Orbulina universa*, as this species is among the most comprehensively studied in terms of test calcification, physiology, and foraminiferal–algal symbiosis, e.g., [15–21]. Although it is not widely used for seawater palaeothermometry, *O. universa* has been the subject of three Mg/Ca thermometer calibration studies [4,13,14] that report temperature-dependent increases in bulk test Mg/Ca composition consistent with other planktonic species (i.e., $9\pm 1\% /^\circ C$). However, the two laboratory-based studies [13,14] are notable for also suggesting that bulk test Mg/Ca compositions may increase by as much as $6\pm 3\%$ for each 0.1 unit decrease in seawater pH (or equivalent change in seawater carbonate ion concentration).

2. Background

Living *O. universa* are ubiquitous in temperate through tropical oceans [22–26], inhabiting the euphotic zone (upper 100 m) where light levels are sufficient for photosynthetic activity of their many thousands of algal symbionts [15]. *O. universa* is unique among modern planktonic foraminifera as it develops a final, large, spherical chamber (400–1000 $\mu$m diameter) that completely envelopes the smaller, spirally arranged, juvenile chambers [17–19]. This final chamber is initially thin-walled but thickens over a period of 3 to 7 days until it dominates the test mass (>90–95%) [17–19]. Final chamber calcification commences upon an organic template (known as the primary organic membrane or POM), which remains

![Fig. 1. SEM images of a typical adult *O. universa* test (A) and test wall section (B). Pores, internal layering, and location of the POM near the inside wall, are clearly visible. Protrusions on test outer surfaces are the remnant bases of spines that would have originally extended 1.5–2.5 mm from the test surface [19]. Spines dampen buoyancy-driven vertical migration, are used to catch prey, and provide a framework on which to host algal symbionts [26]. Ten LA-ICPMS analysis pits and a larger hole formed by a tungsten needle can be seen in (A). Note scale bars.](image-url)
near the inside wall, as most calcite is precipitated on the outside rather than inside surface of the test (Fig. 1) [17,19]. Very thick-walled tests (>30 μm) often have a characteristic, coarsely crystalline, calcite crust that can comprise more than half the test mass [18]. These crusts are notable for being common on fossil tests recovered from seafloor sediments but absent from tests collected live at depths <100 m [25] and <300 m [23], thus suggesting they form immediately prior to reproduction and ensuing death, in water deeper than 300 m [23].

3. Experimental Method

Tests of *O. universa* (>400 μm diameter) were hand-picked from seafloor surface sediments (0–1 cm) sampled by gravity core (FR10/95-GC14) on the margin of the Exmouth Plateau in the eastern Indian Ocean (20°02.71′ S, 112°39.73′ E) at 997 m water depth. The mean annual sea surface temperature at this location is 25.5 °C, with a seasonal range between 24 and 28 °C (Fig. 2) [27]. The oxygen isotope stratigraphy of this core places the Last Glacial Maximum at ~30-cm depth and commencement of the Holocene at around 10-cm depth [28], consistent with many other low sedimentation rate cores offshore Western Australia. A Late Holocene to modern age for the studied core–top sample is supported by uncalibrated AMS radiocarbon dates of 13,050±110 years at 23- to 24-cm depth, and 18,300±190 years at 23–24 cm (both AMS dates were obtained on planktonic foraminifera).

Selected *O. universa* tests were cleaned by gentle ultrasonication in reagent grade methanol to remove adhering detrital material. Individual tests were then broken into large fragments, some of which were mounted in epoxy for electron microprobe analysis and others on glass slides using carbon tape to facilitate laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) depth profiling and viewing of test surfaces and internal ultrastructure by scanning electron microscopy (SEM; Fig. 1).

The distributions of Mg and Ca within tests walls were determined by laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) and by electron microprobe analysis. A high-resolution LA-ICPMS technique that employs a pulsed ArF excimer laser (λ=193nm) coupled to an Agilent 7500s inductively coupled plasma mass spectrometer (ICPMS) was used to generate depth profiles through test walls [29]. The spatial and depth resolution of this technique was optimised by ablating small diameter (30 μm) spots at 3 laser pulses s⁻¹, in an ablation cell with a mean particulate residence time of ~0.35 s, as described previously [29]. Best results were obtained by ablating outward through the test wall from the inner surface due to the effects of surface topography [29]. A small number of isotopes and trace elements (24Mg, 25Mg, 27Al, 43Ca, 44Ca, 55Mn) were measured during each depth profile analysis which required between only 20 and 60 s to acquire.

For electron microprobe analysis, whole tests and test fragments were mounted in epoxy, then sectioned, flattened, and polished to expose test walls in cross-section. Mg and Ca were mapped on a 0.5 μm grid spacing in representative wall cross-sections of each test, by wavelength-dispersive methods using a Cameca SX100 electron microprobe operating at a 15 kV accelerating voltage and 10 nA beam current.
Mg and Ca intensities were measured using TAP and PET crystals and acquired for 0.5 s at each grid position, requiring analysis times of several hours for a typical, 50×50 μm area. Mg/Ca profiles were constructed by integrating measured Mg/Ca ratios parallel to the banding direction in the Mg/Ca intensity ratio maps, and quantified by referencing to Mg/Ca profiles measured in the same tests by LA-ICPMS and applying a constant calibration factor to obtain consistent Mg/Ca values. It should be noted that the time to generate these Mg/Ca profiles is two orders of magnitude greater than required by LA-ICPMS. However, an advantage of the electron microprobe method is that features observed in any Mg/Ca profile can be unambiguously linked to specific parts of the test. For example, the position of the primary organic membrane (POM) is identified in all tests by coincidence of a low-intensity band in Ca maps and a narrow band of low brightness in backscattered electron images (Fig. 3).

4. Results

Mg/Ca maps reveal the development of striking compositional layering in all tests. This layering comprises alternating, low and high Mg/Ca bands that lie parallel to the curved inner and outer surfaces of the test wall (Fig. 4). The LA-ICPMS depth profiles record variations in Mg/Ca through test walls that are consistent with profiles that have been derived from the electron microprobe maps by integrating Mg and Ca intensities along the growth banding (Fig. 5).

The POM is always colocated with a band of relatively low Mg/Ca calcite (3.5–5.5 mmol/mol). Between three and six pairs of alternating low and high Mg/Ca bands occur between the POM and the outer surface, across which the amplitude of Mg/Ca variation is typically 30–50% but occasionally >100 and as much as 200%. In virtually all tests analysed, this finely spaced banding is superimposed upon a broader increase in Mg/Ca that is developed outward from the POM over the first three or four pairs of low and high Mg/Ca bands. A broad outer layer, with very low Mg/Ca (~3 mmol/mol) and no fine-scale banding, is developed on thick-walled tests that display characteristic evidence of outer crust formation (see B and G in Fig. 5). A prominent, high Mg/
5. Discussion

The observed number of paired low and high Mg bands between the POM and test outer surfaces match the number of days that have been reported to occur between final chamber formation and reproduction by *O. universa* in laboratory culture experiments (typically 3–5 days [19], range=2 to 12 with an average of 5.4 days [17]). Accordingly, we interpret each paired low and high Mg band to be the result of a diurnal cycle in test calcification. We also suggest that an equivalent record of Mg/Ca variation may also be reproduced between the POM and the inside test surface, but is condensed into a scale beyond the spatial resolution of our microanalysis techniques (Fig. 5). It is notable that the development of compositional layering has been documented recently in thick-walled benthic foraminifera [30].

The large Mg/Ca variation that is observed from the POM to the outer test surface provides a continuous record of changing calcification conditions from commencement to the end of final chamber growth in *O. universa*. If the Mg/Ca composition of calcite precipitated by *O. universa* is assumed to be temperature controlled and to increase by between 8.5% and 10.5% °C−1 [4,13,14], then the amplitude of Mg/Ca variation within individual tests (which range from a factor of 2.7 up to 3.5) requires their growth over a range of temperatures that span at least 9.5 °C and as much as 14.7 °C (Fig. 5). If the low Mg/Ca outer crusts that are present on some tests are excluded from consideration, the smallest required temperature range is reduced but only by <1 °C. These implied changes in calcification temperature exceed that within the euphotic zone at the sample site, where the modern mean annual range between 0 and 100 m is only 3.5 °C and the maximum seasonal range is 6 °C (Fig. 2) [27].

Absolute calcification temperatures based on the measured Mg/Ca profiles in each test, have been calculated using the three available Mg/Ca thermometer calibrations for *O. universa* (see Fig. 5) [4,13,14]. In comparing these below with modern seawater temperatures at the sampling site, we make the assumption that similar conditions existed during the Late Holocene. Bulk test Mg/Ca compositions, estimated by integrating Mg/Ca profile compositions from the inner to outer surface (but excluding any low Mg/
Ca outer crust where present), correspond to temperatures between 20.8 and 25.6 °C (mean=22.9 ± 2.0 °C) using the Russell et al. [14] calibration, between 18.4 and 23.9 °C (mean=20.5 ± 2.1 °C) using the Lea et al. [13] calibration, and between 23.5 and 31.8 °C (mean=28.5 ± 2.0 °C) using the Anand et al. [4] calibration. The higher temperatures obtained using the Anand et al. [4] calibration correspond to modern sea surface and mixed layer temperature variation at the sample site, whereas the other thermometers return temperatures more in keeping with the upper thermocline, albeit below the euphotic zone (i.e., depths>100 m, cf. Fig. 2) in the case of the Lea et al. [13] calibration. However, the Anand et al. [4] calibration indicates maximum calcification temperatures that range from 31 up to 38 °C, based on the highest Mg/Ca

Fig. 5. Mg/Ca profiles measured by LA-ICPMS (blue) and electron microprobe (red) through representative O. universa test walls. The wall inner surface coincides with the origin on the x-axis. Panels (B2) and (B) show a consistently oriented electron microprobe Mg/Ca map and LA-ICPMS Mg/Ca depth profile through the same test (#B in Fig. 4). Right-side axes show test calcification temperatures estimated using [14]. Alternative temperature scales in panels (D) and (E) are calculated using [4,13]. Numerals indicate the sequence of daily growth bands in each test. The higher Mg/Ca composition measured by electron microprobe for the outer crusts [see panels (B) and (G)] and high Mg/Ca values occurring at both ends of the LA-ICPMS profiles reflect contributions from Mg-rich contaminants within pores and on the inner and the outer test surfaces, respectively. The light blue shaded area shows the average annual temperature range within the euphotic zone (i.e., between 0 and 100 m depth), and the orange line shows the summer sea surface temperature maximum at the sample site [27].
Ca profile values observed within individual tests. These temperatures are unacceptably high, even for tropical oceans, and significantly exceed modern sea surface temperatures at the sampling site (i.e., 24 to 28 °C, see Fig. 2) [27]. The other two Mg/Ca thermometers return maximum temperature estimates that lie between 23 and 30 °C (using [13]) and between 25 and 31 °C (using [14]). These ranges also extend above modern sea surface temperatures at the core site, but only by a few degrees and are in keeping with independent sea surface temperature estimates for the Fr10/95-GC14 core during the Holocene (i.e., 25.5 to 28 °C, see Fig. 11 in [28]). The low-Mg/Ca outer crusts that are developed on some tests have compositions indicative of much lower calcification temperatures, ~9.5 °C (using [13]) and ~13–14 °C (using [14]), consistent with water depths that have been previously suggested for the development of final outer crusts on *O. universa* (i.e., >300 m, cf. Fig. 2) [23]. Relatively low temperatures are also indicated for the initial stage of final chamber growth, during and immediately following POM development, with estimated values ranging between 11 and 16.5 °C (using [13]) and between 15.2 and 19.5 °C (using [14]). These temperatures correspond to depths in the range 250–400 m (using [13]) and 180–280 m (using [14]), which lie well below the euphotic zone habitat of *O. universa* (cf. Fig. 2).

The daily Mg/Ca banding that is observed in each test (see Fig. 5) could be attributed to temperature changes arising from a diurnally regulated cycle of ascent and descent though the water column. While the occurrence of daily vertical migration has been speculated previously, conclusive evidence has been found lacking in plankton tows studies that have specifically sought to test this hypothesis [25,31]. We further note that the amplitude of Mg/Ca variation within individual foraminifers would require improbably large, daily changes in habitat depth that extend well below acceptable habitat for *O. universa*, particularly during the earliest stages of final chamber growth (cf. Figs. 2 and 5). Both this and the large range of apparent calcification temperatures within individual tests strongly suggest that factors additional to temperature are controlling the Mg/Ca composition of calcite precipitated by *O. universa*.

pH microsensor measurements and theoretical models have shown seawater pH and carbonate saturation state to be modified in a diffusive boundary layer that surrounds planktonic foraminifera and to be modulated within this microenvironment by the cycle of daytime photosynthetic activity and nocturnal respiration of algal symbionts [21,32,33]. This produces a large diurnal range in pH at the test surface that has been shown to vary from pH 7.9 (dark) to pH 8.8 (light) [21]. Furthermore, the reduction in calcite saturation state that occurs at lower pH accounts for the twofold to threefold lower nighttime versus daytime calcification rate that has been documented in *O. universa* [20]. Together with the 3–6% increase in bulk test Mg/Ca composition that occurs with each 0.1 pH unit lowering of external seawater pH [13,14], this implies nighttime calcification should produce thinner bands that are 35 to 80% more Mg-rich than formed during the day. This appears to be in keeping with the occurrence of distinctly thinner high Mg/Ca bands interleaved between thicker low Mg/Ca bands in some tests (see Figs. 4 and 5) and the amplitude of Mg/Ca variation (30–60%) that is observed across most daily bands in many tests (Fig. 5). The much larger daily Mg/Ca fluctuations (up to a factor of three) that are occasionally observed could reflect either: (1) pH changes exceeding 1 log unit; (2) underestimation of the effect of pH on Mg/Ca composition of *O. universa* by existing experimental data; or (3) amplification of pH effects by modest changes in calcification temperature due to limited vertical migration within the uppermost thermocline at depths <100 m.

The reason for the broad and large Mg/Ca increase (by 200–250%) that occurs outward from the POM over the first three or four daily growth cycles in most tests is not clear. Temperature is also an inadequate explanation for this increase as the required change, 6.6 to 10.8 °C (calculated using [14]), exceeds the temperature range within the euphotic zone habitat of *O. universa*, and would require final chamber growth to commence at depths greater than 200 m (cf. Fig. 2). Alternatively, the required reduction in pH is at least 1.1 to 1.5 log units, if the largest of the available Mg/Ca–pH relationships is applied [13,14]. If indeed pH is responsible, several plausible mechanisms exist for systematically reducing the pH at which test calcification occurs including: (1) reduced efficiency of symbiont photosynthesis due to decreasing light levels as tests thicken with age and sink into deeper water;
(2) increased respired CO₂ loading of the foraminifer’s microenvironment due to growth of the host foraminifer; and (3) changed location and intensity of CO₂ drawdown at the test surface due to changing number, density and position of algal symbionts on spines [15]. The reversal toward lower Mg/Ca that usually occurs after about the fourth or fifth daily cycle could record the overriding of pH effects by a reduction in temperature, as foraminifera begin to sink and enter the upper thermocline. Continued sinking into much colder and deeper water (>300 m), accelerated by loss of spines prior to reproduction [26], accounts for the very low Mg/Ca compositions of the final outer crusts.

Finally, we note that variation in the vitality of individual foraminifers and their algal symbionts, or variation in the number and location of symbionts on individual foraminifers, could explain the unusually high variance reported for O. universa test Mg/Ca compositions that have been otherwise cultured under the same temperature conditions [13]. Furthermore, given temperature and seawater pH (or carbonate ion composition) also affect the stable-isotope composition of planktonic foraminifera [34–36], including O. universa [16], we speculate that similar diurnal banding in oxygen, carbon, and boron isotopes might also occur in O. universa.

6. Summary

High spatial resolution microanalysis results indicate the Mg/Ca composition of O. universa tests is acutely sensitive to influences that change the biological activity (specifically respiration and photosynthesis) of foraminifera and their algal symbionts. Accordingly, palaeoseawater temperatures could be unreliable if bulk test compositions of O. universa are not in all situations consistently affected by these influences. It is conceivable that biases could be introduced by variations in light intensity with latitude, season, cloudiness, and/or depth within the euphotic zone, or other factors that affect the vitality of either symbiont or foraminifer, and impact on photosynthetic activity and respiration. An important question for palaeocean and palaeoclimate reconstruction is whether, and to what extent, the Mg/Ca compositions of other planktonic foraminifer species that are widely used for palaeoseawater thermometry are subject to similar influences.

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