# Relationship between $\delta^{18}$O and minor element composition of *Terebratalia transversa*

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ABSTRACT: With their extensive fossil record and shells of stable low-Mg calcite, rhynchonelliform brachiopods are attractive sources of climate information *via* seawater temperature proxies such as stable oxygen isotope composition. In *Terebratalia transversa* (Sowerby) there is a progression towards oxygen isotope equilibrium in the calcite of the innermost secondary layer. This study confirms the lack of any vital effects influencing oxygen isotope composition of *T. transversa*, even in specialised areas of the innermost secondary layer. Calcite Mg/Ca ratio is another potential seawater temperature proxy, that has the advantage of not being influenced by salinity. Mg concentrations measured by electron microprobe analyses indicate that there is no concomitant decrease in Mg concentration towards the inner secondary layer, associated with the progressive shift towards oxygen isotope equilibrium. Mg distribution is heterogeneous throughout the shell and correlates with that of sulphur, which may be a proxy for organic components, suggesting that some of the Mg may not be in the calcite lattice. It is essential therefore, to determine the chemical environment of the magnesium ions to avoid any erroneous temperature extrapolations in brachiopods or any other calcite biomineral.

KEY WORDS: brachiopods, calcite, magnesium, oxygen isotopes, sulphur, vital effect.

The extensive fossil record of brachiopods makes them attractive sources of climate information *via* seawater temperature proxies such as the stable oxygen isotope composition of the calcite shell. This approach originates from the proposal (Urey 1947) that the abundance of $^{18}$O in marine biogenic carbonates may be used as a proxy for the temperature of the ambient seawater. Lowenstam (1961) tested this claim on the brachiopods and concluded that they precipitate shell calcite in isotopic equilibrium with seawater. An exception to the widely accepted conclusion was noted (Lepzelter *et al.* 1983) in specimens from cold water habitats where disequilibrium occurred. A wider concern came from Popp *et al.* (1986) with the suggestion that oxygen isotope fractionation in brachiopod shell calcite may be due to biology ('vital effects') rather than environmental conditions. Carpenter & Lohmann (1995) further examined the isotopic equilibrium situation of the brachiopods, maintaining that if oxygen isotope fractionation in marine calcite produced by other phyla is influenced by vital effects (McConnaughey 1989; Ortiz *et al.* 1996; Bohm *et al.* 2000), then more data are required for brachiopods. Carpenter & Lohmann (1995) warned against the use of the brachiopod shell primary layer in isotope measurements and recommended use of the secondary layer. The only exceptions to this pattern of suitability were species of *Thecidellina*, since they have no defined secondary layer (Williams 1973), and species of *Stetothyris* from cold Antarctic waters. Adlis *et al.* (1985, 1988) also warned against the use of brachiopod primary layer calcite, due to the greater susceptibility of primary layer calcite, to diagenetic alteration when compared to secondary layer calcite fibres displayed in Late Pennsylvanian brachiopods.

Buening & Spero (1996) used $^{18}$O content within growth bands in the shell to recognise El Niño warming events,

concluding that brachiopod shell $\delta^{18}$O content is a useful record of environmental data. A study of $^{18}$O composition of brachiopod (species unspecified), bivalve and gastropod shells by Rahimpour-Bonab *et al.* (1997) concluded that brachiopod shells were often isotopically heavier than equilibrium values. James *et al.* (1997) measured the oxygen isotope composition of four terebratulid species and concluded that the brachiopod shell does reflect variations in ambient seawater temperature. Curry & Fallick (2002) reported different $\delta^{18}$O values from dorsal and ventral valves of *Calloria inconspicua* (Sowerby) from Otago Shelf in New Zealand. Brand *et al.* (2003) brought together much of the published data on brachiopod shell isotope composition along with their own data to devise an 'oxygen isotope equilibrium incorporation test'. Despite the fairly liberal criteria, some brachiopods fail this test or produce ambiguous results. The systematic study by Parkinson *et al.* (2005) included 122 brachiopods all collected live with representatives from all extant calcite-precipitating genera from eight different localities, providing a range of environments and latitudes. The conclusions supported those of Carpenter & Lohmann (1995) in that the $\delta^{18}$O values from the fibrous secondary layer or prismatic tertiary layer of articulated Terebratulida and Rhynchonellida species were in oxygen isotope equilibrium with ambient seawater. $\delta^{18}$O values were relatively unaffected by shell specialisation. The primary layer of brachiopod shells is isotopically light in terms of oxygen and carbon, with the exception of the rhynchonellid *Notosaria nigricans*. Therefore, inclusion of primary layer material in any isotope measurements will result in extrapolation to erroneously elevated temperatures (cf. Curry & Fallick 2002). The Thecideidine *Thecidellina barretti* (Davidson), composed of primary layer, was not deemed suitable for isotope

measurements, and neither was the craniid *Novocrania anomala* (Müller).

Auclair *et al*. (2003) studied *Terebratalia transversa* (Sowerby) in detail, measuring the carbon and oxygen isotope composition from the outside to the inside of the shell. Their study indicated that there is no abrupt switch over to equilibrium values at the initiation of the secretion of the secondary layer; but instead, a progression towards oxygen (and carbon) isotope equilibrium conditions towards the innermost secondary layer.

In other marine biogenic carbonates such as foraminiferan tests (Elderfield & Ganssen 2000; Lear *et al*. 2000; Raja *et al*. 2005; Toyofuku & Kitazato 2005), coccoliths (Stoll *et al*. 2001) and coralline algae (Ries 2004, 2006), Mg/Ca ratios record the temperature of ambient seawater. The advantage is that, unlike carbonate $\delta^{18}O$, the magnesium concentration in the calcite lattice is not influenced by water $\delta^{18}O$ and its proxy, salinity (Klein *et al*. 1996). Bentov & Erez (2005) described excellent biological control over magnesium concentration in the chamber wall of foraminiferan tests, explaining that environmental factors such as water temperature can control magnesium concentration within the range set by biology. Whilst the Rhynchonelliformea have shells of low-Mg calcite, little work has been done on the magnesium concentration of brachiopod shells to determine whether or not the magnesium concentration records seawater temperature. Stanley (2006) presented changes in seawater magnesium concentration throughout the Phanerozoic as a factor influencing the composition of biominerals. England *et al*. (2007) reported differences in the magnesium content and distribution in shells of *Terebratulina retusa* compared to *Novocrania anomala* when they were collected from the same site, and thus seawater of identical Mg concentration with the calcite likely precipitated at the same temperature. Whilst *N. anomala* has 2·55 wt % magnesium throughout the shell, *T. retusa* shells have a lower overall magnesium content. In *T. retusa*, however, the magnesium is not evenly distributed, with the highest concentration (3·5 wt %) in the primary layer and a maximum concentration of 1·5 wt % in the secondary layer, with an average secondary layer concentration of 1 wt %. The contrast in magnesium distribution is not restricted to inter-subphyla comparisons, but is evident within the subphylum Rhynchonelliformea. Buening & Carlson (1992) noted changes in magnesium content associated with ontogeny in *Terebratulina unguicula* (Carpenter) and *Terebratalia transversa*, where the magnesium concentration in the umbo is at least double that in the shell anterior. Lowenstam (1961) suggested that magnesium and strontium vary with brachiopod taxonomy. Buening & Carlson (1992) demonstrated that, in addition to ontogenetic influences, taxonomic differences do occur, with the demonstration that *Terebratulina unguicula* has a higher magnesium content than *Terebratalia transversa*. Bates & Brand (1991), studying Middle Devonian brachiopods, concluded that while *Athyris spiriferoides* (Eaton), *Mediospirifer audacula* (Conrad) and *Mucrospirifer mucronatus* (Conrad) controlled the incorporation of magnesium, strontium and sodium into their shell, their isotopic composition was free from any vital effects. Lee *et al*. (2004) used laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) to demonstrate the symmetrical distribution of Sr, Na, Mg, Mn, B and Ba in modern specimens of *Magellania flavescens* (Lamarck) and *Terebratulina septentrionalis* (Couthouy). They then exploited this symmetrical distribution of elements to determine that the minor and trace element composition of the well preserved Devonian brachiopod *Independatrypa lemma* recorded original ambient seawater composition (Lee *et al*. 2004).

This present study concentrates on *T. transversa*, since detailed isotopic composition throughout the shell secondary layer is available. The $\delta^{18}O$ composition is considered in detail in the context of shell structure and specialisation, and the magnesium concentration is presented in the same context and spatial resolution as the isotope measurements of Auclair *et al*. (2003). If progression towards isotopic equilibrium conditions reported by Auclair *et al*. (2003) is solely a result of the kinetics of crystal growth with decreasing growth rate as the organism ages, then magnesium incorporation may decrease in the same manner, with magnesium being excluded from calcite growing at a slower rate. This hypothesis is tested, providing fundamental data that are essential before Mg/Ca ratios can be considered as a temperature proxy in brachiopods. Jimenez-Lopez *et al*. (2004) demonstrated the influence of high magnesium content (>10 mol %) on $\delta^{18}O$ values in synthetic magnesium calcite, and called for further work to clarify the influence of magnesium co-precipitation on oxygen isotope fractionation. Sulphur concentration and distribution has been included in the present study in order to determine whether or not the organic content influences either the isotope or trace element proxy for seawater temperature.

## 1. Materials

Specimens of *Terebratalia transversa* were collected from Puget Sound, near Friday Harbor, Washington USA, 48.5°N, 122°W.

## 2. Methods

*T. transversa* valves were disarticulated and the pedicle and soft tissues removed using dental tools. Valves were cleaned in an ultrasonic bath using aqueous solution of sodium hypochlorite (1% v/v) and thoroughly rinsed in Milli Q™ water.

Samples of shell material were extracted for stable isotope analyses from cleaned valves using a dental drill under a binocular microscope. Material was taken from easily identified morphological features and ultrastructural layers of both ventral and dorsal valves. All samples were pre-treated using low-temperature plasma-ashing for 4 h to remove volatile organic contaminants. Stable isotope analyses were carried out at the Scottish Universities Environmental Research Centre (SUERC) using a VG ISOGAS Prism II mass-spectrometer fitted with an ISOCARB automated carbonate preparation system. Precision was monitored by incorporating internal laboratory marble standards within each batch of analyses. Standard material was calibrated to the International Atomic Energy Agency's (IAEA) intercomparison material IAEA-CO-1 Carrara marble. Reproducibility of the standard was better than 0·1‰ at 1σ. Each sample was replicated three times. Replication was less than 0·2‰ at 1σ. Results were corrected for $^{17}O$ effects (Craig 1957) and presented in standard $\delta^{18}O$ and $\delta^{13}C$ notation permil (‰) relative to VPDB international standard.

Isotopic temperatures were calculated from $\delta^{18}O$ values using the isotopic temperature equation (Anderson & Arthur 1983):

$$T\,(°C) = 16\cdot0 - 4\cdot14\,(\delta^{18}O_{calcite} - \delta^{18}O_{seawater}) + 0\cdot13\,(\delta^{18}O_{calcite} - \delta^{18}O_{seawater})^2$$

For electron probe microanalysis (EPMA), polished blocks of *T. transversa* valves were prepared. *T. transversa* shell samples were cut and mounted in araldite resin blocks and polished through a series of grinding and polishing discs.
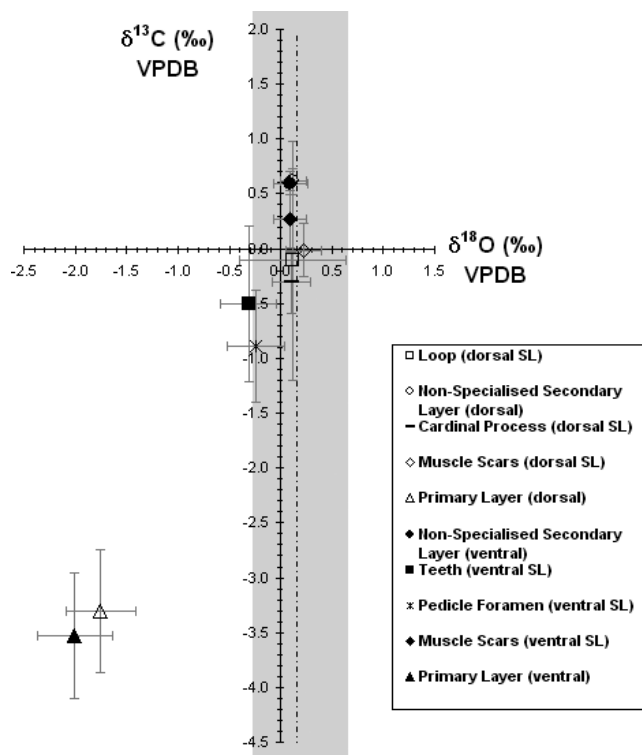
**Figure 1** $\delta^{18}O$–$\delta^{13}C$ cross-plot of *Terbratalia transversa* (Sowerby). Cross-plot includes all areas analysed from both dorsal and ventral valves. Data points represent mean values; error bars indicate $1\sigma$. Dashed box indicates oxygen isotope equilibrium parameters with the mean value indicated by the dashed line. Open and filled symbols indicate samples from dorsal and ventral valves respectively: Circles= non-specialised secondary layer; diamond=muscle scars; triangles= primary layer; –=cardinal process; small filled square=teeth; X=pedicle foramen; open square=loop; large filled square=bulk shell.

Initially the sample surface was ground down using diamond impregnated papers at 74 µm and then 20 µm; diamond slurry at 8 µm then 6 µm are followed by a compound diamond pad at 6 µm then 3 µm. The polishing stages were performed with alpha aluminium oxide at 1 µm and 0·3 µm, with a final treatment with 0·06 µm colloidal silica on a short nap disc. EPMA of carbon coated polished samples used a Cameca SX50 Electron Microprobe equipped with three wavelength dispersive X-ray spectrometers. Quantitative analyses of magnesium and sulphur were obtained using 15 kV and 10 nA and the beam was defocused to 10 µm in order to prevent beam damage. Mineral standards were used and matrix corrections were carried out using a PAP (Pouchon & Pichoir 1984) correction procedure. Spot analyses were carried out on longitudinal sections, with lines of spot analyses evenly spaced from the posterior (A) to the anterior (F). Analyses were every 10 µm across from the outer shell (primary layer) to the innermost secondary layer. Paired t-test in Excel was used to test the null hypothesis that the concentration of Mg and S at each analysis point coincided by chance.

## 3. Results

Results from isotopic measurements of *T. transversa* are summarised in Figures 1 and 2 and the magnesium and sulphur measurements in Figure 3 and Table 1.

### 3.1. Stable oxygen isotopes of *T. transversa*
Analyses of primary, secondary and specialised secondary layer of *T. transversa* reveal that the oxygen and carbon isotope compositions (Fig. 1) of the primary layer are depleted
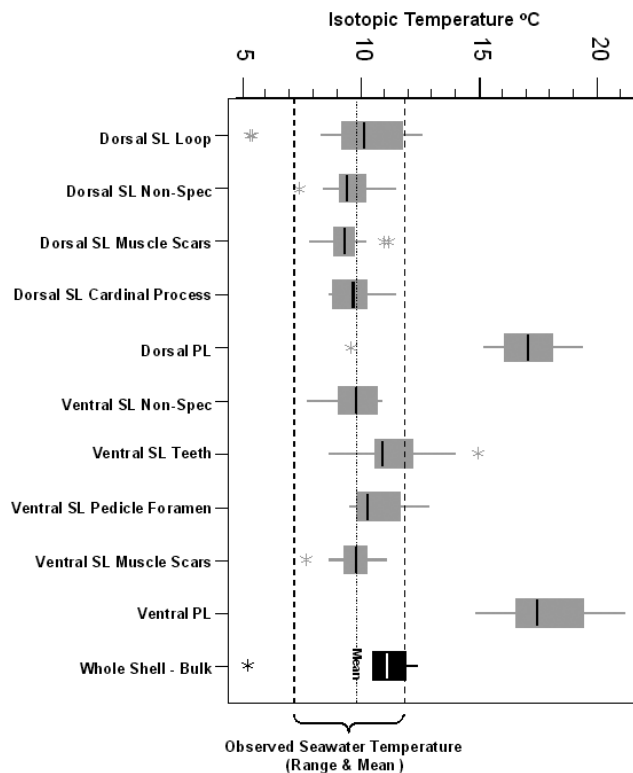


**Figure 2** Boxplot showing the range of isotopic temperatures extrapolated from $\delta^{18}O$ values of all analyses from different regions of the shell of terebratulid brachiopod *Terebratalia transversa* (Sowerby). Comparison is made with the measured seawater temperature range from Friday Harbor (Carpenter & Lohmann 1995).

(isotopically lighter) relative to the secondary layer. The $\delta^{18}O$ values for the primary layer are outside the expected range of values for equilibrium with ambient seawater.

In the analyses of the innermost secondary layer, there is no correlation between $\delta^{18}O$ and $\delta^{13}C$. Samples from the innermost secondary layer are within equilibrium and therefore suitable for interpreting ambient water temperature. There is no difference between the isotopic composition of the dorsal and ventral valves of *T. transversa*. Therefore, whilst metabolic prioritisation of carbon isotopes is apparent in specialised regions of the brachiopod secondary layer (Parkinson & Cusack 2007), the oxygen isotope composition is within equilibrium values, even in specialised regions. The box-plot in Figure 2 presents these data in the context of seawater temperature, indicating the temperatures that would be determined from oxygen isotope composition of the various shell parts and the actual mean water temperature to which *T. transversa* has been exposed. Primary layer alone would suggest temperatures 10°C above the mean ambient water temperature, whilst all secondary layer regions sampled are within the temperature range (Fig. 2). Bulk shell analyses are slightly elevated due to the inclusion of primary layer material. Since the analyses of the secondary layer are all of the innermost part, the isotopic values would be expected to be within equilibrium values according to Auclair *et al.* (2003). The additional important point that is highlighted by this sampling strategy is that, even in specialised regions such as muscle scars, the oxygen isotope composition is within the equilibrium range, indicating a lack of vital effects that might distort the oxygen isotope fractionation.

### 3.2. Magnesium and sulphur distribution in *T. transversa*
Although *T. transversa* has a shell of low Mg-calcite, the magnesium concentration is not constant throughout the shell
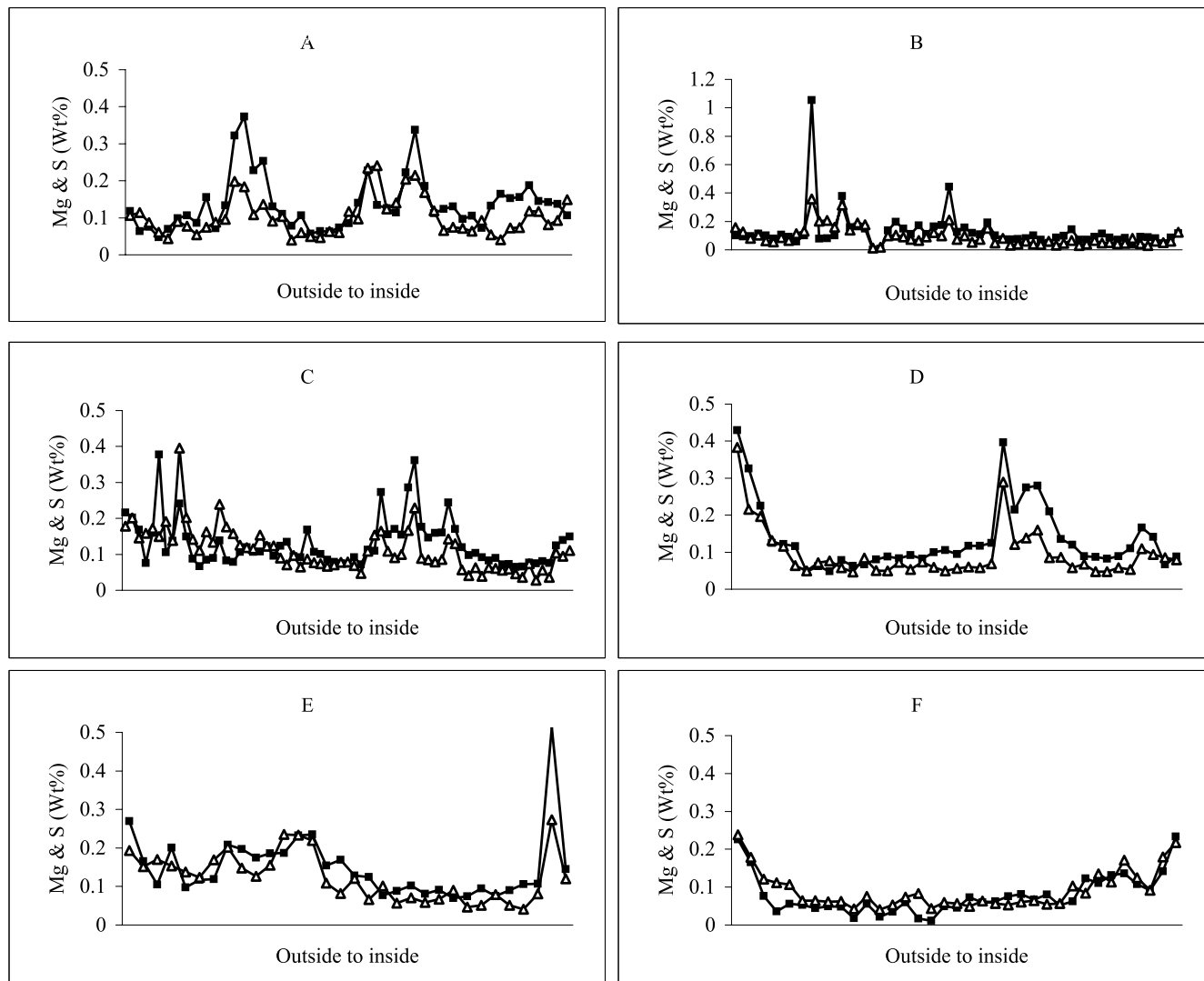
**Figure 3** Electron probe microanalyses (EPMA) of transects from outer to inner dorsal valve of *Terebratalia transversa*. Evenly spaced transects from (A) posterior to (F) anterior with magnesium indicated by filled squares and sulphur by open triangles. Note that B is on a different scale to the other graphs.

(Fig. 3). In each of the six transects depicted in Figure 3, there are regions with elevated magnesium concentrations. Therefore, using electron probe microanalysis to measure magnesium concentrations from one part of the shell, such as the innermost secondary layer, would not represent the mean magnesium concentrations in the shell. There is no evidence in any region of the shell for magnesium concentration decreasing progressively from outer to inner shell. Despite the variation in magnesium and sulphur concentrations throughout each transect, the mean values are fairly constant (Table 1), with the exception of the anterior, where magnesium occurs in lower concentrations that in the rest of the shell. This is in agreement with Buening & Carlson (1992), who found higher magnesium concentrations in the umbo than in the anterior. Paired t-tests rejected the null hypothesis that the concentration of Mg and S at each analysis point in the transects coincided by chance (Table 1). The positive correlation of magnesium and sulphur (Table 1) in each transect strongly suggests that in *T. transversa*, magnesium is associated with sulphur in an organic or inorganic phase and that magnesium, therefore, may not be a true component of the calcite lattice.

## 4. Discussion

The sampling strategy employed here indicates clearly that, for *T. transversa*, the oxygen isotope compositions of the inner-most secondary layer, including the specialised regions, are not influenced by vital effects and thus, the oxygen isotope composition of the innermost secondary layer, including specialised regions, is a proxy for ambient seawater temperature. The progressive trend towards equilibrium throughout the calcite of the secondary layer of *T. transversa* should be investigated in other brachiopods shells to determine whether or not this is a general trend among brachiopods or a peculiarity of *T. transversa*.

Stanley (2006) summarised changes in seawater composition throughout the Phanerozoic, explaining that these changes in seawater chemistry are reflected in the composition of non-skeletal carbonates as well as reef carbonates, whilst other organisms such as bryozoans and bivalve molluscs exert control on biomineral formation such that changes in seawater chemistry may not be recorded directly in their biominerals. The fact that the rhynchonelliform brachiopod *Terebratulina retusa* has a shell of low Mg-calcite, whilst the craniid *Novocrania anomala* has a high Mg-calcite shell when both occur in the same locality (England *et al.* 2007), indicates that rhynchonelliform brachiopods exert such control on Mg incorporation into their shells.

The discovery that magnesium is not constant throughout the shell of *T. transversa* and that it correlates with sulphur concentration suggests that some, if not most of the magnesium, is not in the calcite lattice but is associated with organic

**Table 1** Values for Mg and S concentration from posterior (A) to anterior (F). Mean concentration (wt %) of magnesium and sulphur from each transect (A–F) in Figure 3. t-values comparing Mg & S concentration in each transect in paired t-tests demonstrate that the null hypothesis, that the Mg and S concentrations are similar by chance, should be rejected supporting a statistical correlation between Mg and S concentrations at the analysis points throughout the transects. Mean values of Mg and S concentration throughout each transect and their standard deviation (Std dev) are also presented at the base of Table 1.

| Posterior A | | B | | C | | D | | E | | Anterior F | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Mg | S | Mg | S | Mg | S | Mg | S | Mg | S | Mg | S |
| 0·118 | 0·106 | 0·101 | 0·156 | 0·217 | 0·178 | 0·430 | 0·383 | 0·270 | 0·193 | 0·226 | 0·238 |
| 0·064 | 0·114 | 0·093 | 0·125 | 0·202 | 0·201 | 0·326 | 0·216 | 0·165 | 0·152 | 0·165 | 0·178 |
| 0·076 | 0·087 | 0·095 | 0·080 | 0·168 | 0·146 | 0·225 | 0·197 | 0·105 | 0·170 | 0·077 | 0·121 |
| 0·047 | 0·061 | 0·113 | 0·102 | 0·076 | 0·158 | 0·125 | 0·132 | 0·201 | 0·153 | 0·035 | 0·111 |
| 0·070 | 0·043 | 0·101 | 0·064 | 0·159 | 0·172 | 0·122 | 0·116 | 0·097 | 0·137 | 0·055 | 0·106 |
| 0·098 | 0·090 | 0·078 | 0·056 | 0·377 | 0·150 | 0·116 | 0·063 | 0·117 | 0·124 | 0·053 | 0·064 |
| 0·106 | 0·077 | 0·105 | 0·087 | 0·106 | 0·192 | 0·048 | 0·049 | 0·119 | 0·169 | 0·044 | 0·064 |
| 0·087 | 0·054 | 0·092 | 0·066 | 0·137 | 0·139 | 0·062 | 0·071 | 0·208 | 0·202 | 0·049 | 0·061 |
| 0·156 | 0·074 | 0·057 | 0·114 | 0·241 | 0·395 | 0·048 | 0·076 | 0·197 | 0·148 | 0·048 | 0·062 |
| 0·071 | 0·088 | 0·102 | 0·132 | 0·149 | 0·202 | 0·078 | 0·058 | 0·175 | 0·126 | 0·018 | 0·042 |
| 0·134 | 0·096 | 1·053 | 0·359 | 0·088 | 0·142 | 0·063 | 0·046 | 0·186 | 0·156 | 0·056 | 0·076 |
| 0·322 | 0·198 | 0·078 | 0·201 | 0·067 | 0·111 | 0·067 | 0·084 | 0·187 | 0·235 | 0·022 | 0·040 |
| 0·373 | 0·183 | 0·082 | 0·206 | 0·085 | 0·163 | 0·080 | 0·050 | 0·233 | 0·234 | 0·035 | 0·053 |
| 0·228 | 0·108 | 0·101 | 0·159 | 0·090 | 0·133 | 0·088 | 0·049 | 0·235 | 0·220 | 0·059 | 0·074 |
| 0·253 | 0·136 | 0·378 | 0·316 | 0·138 | 0·239 | 0·084 | 0·072 | 0·154 | 0·109 | 0·017 | 0·084 |
| 0·131 | 0·091 | 0·159 | 0·138 | 0·083 | 0·176 | 0·092 | 0·053 | 0·169 | 0·082 | 0·012 | 0·043 |
| 0·110 | 0·103 | 0·163 | 0·188 | 0·079 | 0·157 | 0·082 | 0·073 | 0·128 | 0·121 | 0·050 | 0·059 |
| 0·078 | 0·039 | 0·148 | 0·177 | 0·107 | 0·125 | 0·100 | 0·059 | 0·125 | 0·066 | 0·045 | 0·057 |
| 0·106 | 0·060 | 0·000 | 0·012 | 0·119 | 0·119 | 0·105 | 0·049 | 0·078 | 0·101 | 0·072 | 0·049 |
| 0·057 | 0·049 | 0·009 | 0·017 | 0·107 | 0·116 | 0·095 | 0·056 | 0·088 | 0·057 | 0·060 | 0·063 |
| 0·064 | 0·046 | 0·136 | 0·099 | 0·108 | 0·153 | 0·117 | 0·060 | 0·102 | 0·071 | 0·062 | 0·057 |
| 0·063 | 0·063 | 0·198 | 0·102 | 0·122 | 0·123 | 0·117 | 0·058 | 0·081 | 0·059 | 0·076 | 0·053 |
| 0·073 | 0·060 | 0·150 | 0·090 | 0·095 | 0·123 | 0·124 | 0·068 | 0·091 | 0·067 | 0·081 | 0·061 |
| 0·085 | 0·117 | 0·110 | 0·073 | 0·123 | 0·090 | 0·396 | 0·289 | 0·070 | 0·090 | 0·067 | 0·064 |
| 0·140 | 0·096 | 0·171 | 0·067 | 0·135 | 0·070 | 0·214 | 0·120 | 0·074 | 0·046 | 0·080 | 0·055 |
| 0·227 | 0·234 | 0·110 | 0·090 | 0·095 | 0·096 | 0·275 | 0·139 | 0·095 | 0·051 | 0·052 | 0·057 |
| 0·134 | 0·240 | 0·165 | 0·121 | 0·092 | 0·065 | 0·279 | 0·160 | 0·075 | 0·079 | 0·063 | 0·103 |
| 0·126 | 0·123 | 0·173 | 0·099 | 0·168 | 0·086 | 0·210 | 0·085 | 0·091 | 0·051 | 0·123 | 0·085 |
| 0·114 | 0·140 | 0·444 | 0·209 | 0·106 | 0·077 | 0·135 | 0·086 | 0·106 | 0·041 | 0·110 | 0·135 |
| 0·223 | 0·204 | 0·126 | 0·074 | 0·101 | 0·073 | 0·120 | 0·058 | 0·107 | 0·081 | 0·131 | 0·113 |
| 0·338 | 0·214 | 0·156 | 0·098 | 0·085 | 0·066 | 0·089 | 0·067 | 0·513 | 0·273 | 0·136 | 0·171 |
| 0·185 | 0·168 | 0·117 | 0·052 | 0·078 | 0·070 | 0·087 | 0·047 | 0·145 | 0·120 | 0·108 | 0·125 |
| 0·112 | 0·119 | 0·108 | 0·074 | 0·075 | 0·078 | 0·082 | 0·047 | | | 0·092 | 0·092 |
| 0·124 | 0·066 | 0·191 | 0·152 | 0·074 | 0·079 | 0·089 | 0·058 | | | 0·142 | 0·180 |
| 0·131 | 0·074 | 0·100 | 0·048 | 0·092 | 0·068 | 0·110 | 0·053 | | | 0·233 | 0·217 |
| 0·096 | 0·071 | 0·072 | 0·081 | 0·072 | 0·047 | 0·166 | 0·109 | | | | |
| 0·106 | 0·064 | 0·073 | 0·032 | 0·112 | 0·109 | 0·142 | 0·093 | | | | |
| 0·073 | 0·093 | 0·075 | 0·042 | 0·109 | 0·153 | 0·067 | 0·085 | | | | |
| 0·133 | 0·054 | 0·080 | 0·060 | 0·273 | 0·164 | 0·088 | 0·079 | | | | |
| 0·164 | 0·040 | 0·100 | 0·042 | 0·155 | 0·109 | | | | | | |
| 0·153 | 0·072 | 0·069 | 0·038 | 0·170 | 0·091 | | | | | | |
| 0·155 | 0·073 | 0·047 | 0·060 | 0·155 | 0·099 | | | | | | |
| 0·187 | 0·118 | 0·086 | 0·034 | 0·285 | 0·166 | | | | | | |
| 0·145 | 0·117 | 0·099 | 0·044 | 0·362 | 0·229 | | | | | | |
| 0·142 | 0·081 | 0·143 | 0·065 | 0·177 | 0·089 | | | | | | |
| 0·138 | 0·093 | 0·070 | 0·031 | 0·146 | 0·085 | | | | | | |
| 0·107 | 0·149 | 0·072 | 0·040 | 0·160 | 0·079 | | | | | | |
| | | 0·091 | 0·066 | 0·161 | 0·086 | | | | | | |
| | | 0·112 | 0·049 | 0·244 | 0·143 | | | | | | |
| | | 0·086 | 0·050 | 0·170 | 0·129 | | | | | | |
| | | 0·071 | 0·042 | 0·119 | 0·057 | | | | | | |
| | | 0·084 | 0·045 | 0·097 | 0·040 | | | | | | |
| | | 0·035 | 0·080 | 0·104 | 0·063 | | | | | | |
| | | 0·091 | 0·043 | 0·092 | 0·039 | | | | | | |
| | | 0·089 | 0·030 | 0·083 | 0·063 | | | | | | |
| | | 0·080 | 0·060 | 0·089 | 0·061 | | | | | | |
| | | 0·044 | 0·051 | 0·072 | 0·056 | | | | | | |
| | | 0·086 | 0·060 | 0·072 | 0·059 | | | | | | |
| | | 0·116 | 0·124 | 0·065 | 0·046 | | | | | | |
| | | | | 0·065 | 0·036 | | | | | | |
| | | | | 0·076 | 0·074 | | | | | | |
| | | | | 0·075 | 0·028 | | | | | | |
| | | | | 0·080 | 0·056 | | | | | | |
| | | | | 0·076 | 0·036 | | | | | | |
| | | | | 0·125 | 0·103 | | | | | | |
| | | | | 0·140 | 0·094 | | | | | | |
| | | | | 0·149 | 0·111 | | | | | | |

| | A | | B | | C | | D | | E | | F | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| t value | 0·000 | | 0·004 | | 0·015 | | 0·000 | | 0·011 | | 0·003 | |
| Mean | 0.14 | 0.10 | 0.13 | 0.09 | 0.13 | 0.11 | 0.14 | 0.10 | 0.15 | 0.12 | 0.08 | 0.09 |
| Std dev | 0.07 | 0.05 | 0.14 | 0.07 | 0.07 | 0.06 | 0.09 | 0.07 | 0.09 | 0.06 | 0.05 | 0.05 |
| | Posterior | | | | | | | | | | Anterior | |

components. Alternatively, some magnesium may be present as an inorganic phase as described by Kampschulte *et al.* (2001). This may result from incorporation of magnesium into a lattice that is distorted by the presence of sulphate ions as demonstrated by Kontrec *et al.* (2004) in synthetic calcite. These possibilities should be investigated, since measurement of magnesium that is not present by ideal substitution would greatly distort estimates of seawater temperature by this proxy. This serves to demonstrate that it is essential to remove any magnesium that is not a true lattice component, or at least quantify the magnesium that is present in the lattice and that associated with organic components using techniques such as X-Ray Absorption Near Edge Spectroscopy (XANES) to determine the chemical environment of the magnesium ions. It is likely that this observation is not only of relevance to the brachiopods and that ideal substitution of magnesium for calcium should not be assumed in any calcite biomineral.

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# 6. References

Adlis, D. S., Grossman, E. L. & Yancey, T. E. 1985. Stable isotope variations in Late Pennsylvanian brachiopods from cyclic sedimentary deposits: paleoenvironmental and diagenetic implications. *Geological Society of America Abstracts with Programs* **17**, 98. Annual Meeting of the Geological Society of America; 28 Oct 1985; Orlando, Florida, USA.

Adlis, D. S., Grossman, E. L., Yancey, T. E. & McLerran, R. D. 1988. Isotope Stratigraphy and Paleodepth Changes of Pennsylvanian Cyclical Sedimentary Deposits. *Palaios* **3**, 487–506.

Anderson, T. F. & Arthur, M. A. 1983. Stable isotopes of oxygen and carbon and their application to sedimentologic and environmental problems. *In* Arthur, M. A., Anderson, T. F., Kaplan, I. R. & Veizer, J. (eds) *Stable Isotopes in Sedimentary Geology. Society of Economic Paleontologists and Mineralogists (SEPM) Short Course Notes*, 1–151. Tulsa, Oklahoma.

Auclair, A.-C., Joachimski, M. M. & Lécuyer, C. 2003. Deciphering kinetic, metabolic and environmental controls on stable isotope fractions between seawater and the shell of *Terebratalia transversa* (Brachiopoda). *Chemical Geology* **202**, 59–78.

Bates, N. R. & Brand, U. 1991. Environmental and Physiological Influences on Isotopic and Elemental Compositions of Brachiopod Shell Calcite – Implications for the Isotopic Evolution of Paleozoic Oceans. *Chemical Geology* **94**, 67–78.

Bentov, S. & Erez, J. 2005. Novel implications on biomineralization processes in foraminifera and implications for Mg/Ca ratio in shells. *Geology* **33**, 841–4.

Bohm, F., Joachimski, M. M., Dullo, W.-C., Eisanhauer, A., Lehnert, H., Reitner, J. & Worheide, G. 2000. Oxygen isotope fractionation in marine aragonite of coralline sponges. *Geochimica et Cosmochimica Acta* **64**, 1695–703.

Brand, U., Logan, A., Hiller, N. & Richardson, J. 2003. Geochemistry of modern brachiopods: applications and implications for oceanography and palaeoeanography. *Chemical Geology* **198**, 305–34.

Buening, N. & Carlson, S. J. 1992. Geochemical investigation of growth in selected Recent articulate brachiopods. *Lethaia* **25**, 331–45.

Buening, N. & Spero, H. J. 1996. Oxygen- and carbon-isotope analyses of the articulate brachiopod *Laqueus californianus*: a recorder of environmental change in the subeuphotic zone. *Marine Biology* **127**, 105–14.

Carpenter, S. J. & Lohmann, K. C. 1995. $\delta^{18}O$ and $\delta^{13}C$ values of modern brachiopod shells. *Geochimica et Cosmochimica Acta* **59**, 3748–64.

Craig, H. 1957. Isotopic standards for carbon and oxygen and correction factors for mass spectrometric analysis of carbon dioxide. *Geochimica et Cosmochimica Acta* **12**, 133–49.

Curry, G. B. & Fallick, A. E. 2002. Use of stable oxygen isotope determinations from brachiopod shells in palaeoenvironmental reconstruction. *Palaeogeography, Palaeoclimatology, Palaeoecology* **182**, 133–4.

Elderfield, H. & Ganssen, G. 2000. Past temperature and $\delta^{18}O$ of surface ocean waters inferred from foraminiferal Mg/Ca ratios. *Nature* **405**, 442–5.

England, J., Cusack, M. & Lee, M. 2007. Magnesium and Sulphur in the Calcite Shells of two Brachiopods, *Terebratulina retusa* and *Novocrania anomala*. *Lethaia* **40**, 2–10.

James, N. P., Bone, Y. & Kyser, T. K. 1997. Brachiopod $\delta^{18}O$ values do reflect ambient oceanography: Lacepede Shelf, southern Australia. *Geology* **25**, 551–4.

Jimenez-Lopez, C., Romanek, C. S., Huertas, F. J., Ohmoto, H. & Caballero, E. 2004. Oxygen isotope fractionation in synthetic magnesian calcite. *Geochimica et Cosmochimica Acta* **68**, 3367–77.

Kampschulte, A., Bruckschen, P. & Strauss, H. 2001. The sulfur isotopic composition of trace sulphates in Carboniferous brachiopods: implications for coeval seawater, correlation with other geochemical cycles and isotope stratigraphy. *Chemical Geology* **175**, 165–89.

Klein, R. T., Lohmann, K. C. & Thayer, C. W. 1996. Bivalve skeletons record sea-surface temperature and $\delta^{18}O$ via Mg/Ca and $^{18}O/^{16}O$ ratios. *Geology* **24**, 415–18.

Kontrec, J., Kralj, D., Brecevic, L., Falini, G., Fermani, S., Noethig-Laslo, V. & Mirosavljevic, K. 2004. Incorporation of Inorganic Anions in Calcite. *European Journal of Inorganic Chemistry* **2004**, 4579–85.

Lear, C. H., Elderfield, H. & Wilson, P. A. 2000. Cenozoic Deep-Sea Temperatures and Global Ice Volumes from Mg/Ca in Benthic Foraminiferal Calcite. *Science* **287**, 269–72.

Lee, X., Hu, R., Brand, U., Zhou, H., Liu, X., Yuan, H., Yan, C. & Chen, H. 2004. Ontogenetic trace element distribution in brachiopod shells: an indicator of original seawater chemistry. *Chemical Geology* **209**, 49–65.

Lepzelter, G. C., Anderson, T. F. & Sandberg, P. A. 1983. Stable isotope variations in modern articulate brachiopods. *American Association of Petroleum Geologists Bulletin* **67**, 500–1.

Lowenstam, H. A. 1961. Mineralogy, $O^{18}/O^{16}$ ratios, and strontuim and magnesium contents of recent and fossil brachiopods and their bearing on the history of the oceans. *The Journal of Geology* **69**, 241–60.

McConnaughey, T. 1989. $^{13}C$ and $^{18}O$ isotopic disequilibrium in biological carbonates: I. Patterns. *Geochimica et Cosmochimica Acta* **53**, 151–62.

Ortiz, J. D., Mix, A. C., Rugh, W., Watkins, J. M. & Collier, R. W. 1996. Deep-dwelling planktonic foraminifera of the northeastern Pacific Ocean reveal environmental control of oxygen and carbon isotope disequilibria. *Geochimica et Cosmochimica Acta* **60**, 4509–23.

Parkinson, D., Curry, G. B., Cusack, M. & Fallick, A. E. 2005. Shell structure, patterns and trends of oxygen and carbon stable isotopes in modern brachiopod shells. *Chemical Geology* **219**, 193–235.

Parkinson, D. & Cusack, M. 2007. Stable oxygen and carbon isotopes in extant brachiopod shells: keys to deciphering ancient ocean environments. *In* Kaesler, R. (ed.) Volume 6: Supplement. *Treatise on Invertebrate Paleontology: Part H, Brachiopoda*, 2522–31. New York & Lawrence, Kansas: Geological Society of America and University of Kansas Press.

Pouchon, J. L. & Pichoir, F. 1984. A new model for quantitative x-ray microanalysis. Part I: Application to the analysis of homogeneous samples *La Reserche Aerospatiale* **5**, 47–65.

Popp, B. N., Anderson, T. F. & Sandberg, P. A. 1986. Textural, elemental and isotopic variations among constituents in middle Devonian limestones, North America. *Journal of Sedimentary Petrology* **56**, 715–27.

Rahimpour-Bonab, H., Bone, Y. & Moussavi-Harami, R., 1997. Stable isotope aspects of modern molluscs, brachiopods, and marine cements from cool-water carbonates, Lacepede Shelf, South Australia. *Geochimica et Cosmochimica Acta* **61**, 207–18.

Raja, R., Saraswati, P. K., Rogers, K. & Iwao, K. 2005. Magnesium and strontium compositions of Recent symbiont-bearing benthic foraminifera. *Marine Micropaleontology* **58**, 31–44.

Ries, J. B. 2004. Effect of ambient Mg/Ca ratio on Mg fractionation in calcareous marine invertebrates: A record of the oceanic Mg/Ca ratio over the Phanerozoic. *Geology* **32**, 981–4.

Ries, J. B. 2006. Mg fractionation in crustose coralline algae: Geochemical, biological, and sedimentological implications of secular variation in the Mg/Ca ratio of seawater. *Geochimica et Cosmochimica Acta* **70**, 891–900.

Stanley, S. M. 2006. Influence of seawater chemistry on biomineralization throughout Phanerozoic time: Paleontological and experimental evidence. *Palaeogeography, Palaeoclimatology, Palaeoecology* **232**, 214–36.

Stoll, H. M., Encinar, J. R. & Alonso, J. I. G. 2001. A first look at paleotemperature prospects from Mg in coccolith carbonate: Cleaning techniques and culture measurements. *Geochemistry, Geophysics and Geosystems* **2**.

Toyofuku, T. & Kitazato, H. 2005. Micromapping of Mg/Ca values in cultured specimens of the high-magnesium benthic foraminifera. *Geochemistry Geophysics and Geosystems* **6**.

Urey, H. C. 1947. The thermodynamic properties of isotopic substances. *Journal of the Chemical Society* **Part 1**, 562–81.

Williams, A. 1973. The secretion and structural evolution of the shell of thecideidine brachiopods. *Philosophical Transactions of the Royal Society of London* **264**, 439–78.