Micro X-ray fluorescence (μXRF) line scanning on Cretaceous rudist bivalves: A new method for reproducible trace element profiles in bivalve calcite

NIELS J. DE WINTER and PHILIPPE CLAEYS
Analytical-, Environmental-, and Geochemistry, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium (E-mail: nidewint@vub.ac.be)

ABSTRACT
The reconstruction of palaeoclimate on a sub-annual scale requires the measurement of chemical proxies in fossil material at a high spatial resolution. While various methods for trace element analysis on the micrometre scale are available, they are usually destructive to the sample or not widely accessible. This study evaluates the performance of a table-top micro X-ray fluorescence scanner in obtaining reproducible trace element profiles on bivalve calcite. Standard calibration and repeatability tests demonstrate the robustness of results obtained with this new generation micro X-ray fluorescence device that uses a comparatively high excitation energy of 30 W. Results show that elemental abundances measured using this fast, readily accessible and non-destructive analytical set-up are reproducible on the μg g⁻¹ level, and therefore suitable for the analysis of the abundance of commonly studied elements in bivalve calcite (for example, Sr, Mn and Fe). Spectra obtained in this study show considerable improvement in terms of signal to noise ratio compared to earlier table-top micro X-ray fluorescence studies. Reliability of the measurements is tested using a conservative detection and quantification limit. Count rates of Ca are used to check for point measurements disturbed by irregularities on the sample surface. Furthermore, the method allows semi-quantitative two-dimensional element mapping, which is a convenient tool for the detection of diagenetic alteration in fossil samples. The method reveals records of Sr/Ca, Fe/Ca and Mn/Ca ratios in a Late Campanian Vaccinites vesiculosus shell, with Sr/Ca ratios showing a trend opposite to Mn/Ca and Fe/Ca. Resulting trace element records are discussed in terms of seasonal cyclicity in the Late Cretaceous and diagenetic alteration of the fossil shell.

Keywords Bivalve, Cretaceous, micro X-ray fluorescence (μXRF), rudist, seasonality, trace element.

INTRODUCTION
In this study, a new method is discussed for obtaining high-resolution elemental abundance records from fossil bivalve shells by using micro X-ray fluorescence (μXRF) scanning. Geochemical profiles through calcium carbonate records are often applied as a method of reconstructing environmental conditions in geological history (e.g. Goreau, 1977; Chivas et al., 1993; Dutton et al., 2002; McDermott, 2004). A variety of carbonate-producing recorders (for example, brachiopods, bivalves, corals and speleothems) are available for this purpose and studies span the entire Phanerozoic (Veizer et al., 1986; Hart & Cohen, 1996; Steuber, 1996; Verheyden et al., 2000). Besides more conventional stable oxygen and carbon isotopes and Mg/Ca ratios, a range of
other geochemical proxies for carbonates have been developed (Gillikin et al., 2008; Schöne et al., 2013; Füllenbach et al., 2015). A multi-proxy approach potentially allows the study of various aspects of the formation history of one archive, and makes it possible to decouple the effects of different parameters on geochemical proxies (for example, δ¹⁸O combined with Mg/Ca to isolate the effect of salinity in coral records; Watanabe et al., 2001). The above advocates the use of multiple geochemical proxies on the same carbonate samples, something which can be difficult to achieve with conventional methods focusing on one geochemical approach (for example, Isotope-Ratio Mass Spectroscopy) or destructive methods that can only be repeated once (for example, digestion-based inductively coupled plasma–mass spectrometry (ICP-MS) and inductively coupled plasma–optical emission spectrometry (ICP-OES) techniques). Table-top µXRF has yielded promising results in archaeological studies (e.g. Castro et al., 2007; Nakano & Tsuji, 2009) and has even provided seasonally resolved records on bivalve calcite (e.g. Kurunczi et al., 2001). However, the spectra obtained for small spot laboratory-based measurements on carbonates only allowed semi-quantitative (in XRF counts per sec) results (e.g. Ziegler et al., 2008; De Winter et al., 2014), because the limited excitation energy of table-top and hand-held systems did not produce the well-resolved point spectra needed to yield repeatable quantified (in mass percent) results. The µXRF method presented in this study overcomes these problems and provides a non-destructive method for measuring quantifiable elemental abundances on sample surfaces, which allows for repeating and re-sampling on the same sample location.

Shells of bivalve molluscs (Mollusca: Bivalvia, Linnaeus 1758) are valuable recorders of information on (palaeo)environmental parameters, and as such have been widely used for (palaeo)environmental reconstruction (e.g. Lazareth et al., 2003; Schöne et al., 2004; Steuber et al., 2005). Bivalves grow by extending their shells incrementally away from the umbones (e.g. Hall et al., 1974), hence recording during their lifetime external environmental conditions at high temporal resolution (Jones, 1980; Weidman & Jones 1994; Sano et al., 2012). Growth rate in bivalves varies significantly on a seasonal scale (Fan et al., 2007). These seasonal changes are reflected in the chemical properties of bivalve shells and are related to variations in environmental parameters and/or the biological response to these parameters (Freitas et al., 2005; Gillikin et al., 2005, 2006). Therefore, elemental abundances measured using µXRF scanning yield valuable information about the seasonal cycle during the growth time of bivalves. This non-destructive method provides a rapid way of obtaining high-resolution proxy records from bivalves and can easily be applied to other recorders of palaeoenvironmental parameters, such as corals, stromatolites and speleothems.

The rudist bivalves (Mollusca: Hippuritida, Newell, 1965) constitute an important group commonly used for the reconstruction of palaeoenvironmental conditions at high resolution in the Cretaceous (e.g. Masse & Philip, 1981; Gili et al., 1995; Steuber, 1995, 1999; Wilson & Opdyke, 1996). Being the dominant carbonate-producing organisms on carbonate platforms during much of the Cretaceous, the shells of rudists are abundant in the fossil record (Ross & Skelton, 1993; Steuber, 2002; Skelton & Gili, 2012). Rudist shells have an outer shell layer of fibrous prismatic calcite while the inner shell layer consists of less diagenetically stable aragonite (Skelton, 1976; Steuber, 1995). In shells of the hippuritids, in common with a few other rudist families, this outer calcite layer is particularly thick compared with other rudist taxa (Skelton, 2013). The prismatic calcite layer in rudist shells is more suitable for palaeoclimatic studies than the inner shell layer, which can contain up to 5% organic matter (Taylor & Layman, 1972; Al-Aasm & Veizer, 1986; Jacob et al., 2008).

The ratios of trace elements, such as Sr, Mg and Ba, with respect to Ca have yielded good results as proxies for palaeoenvironmental conditions (e.g. Weber, 1973; Izuka, 1988; Lazareth et al., 2003; Carré et al., 2006). Culture experiments have shown that these elemental ratios indeed record changes in external factors that influence the growth of bivalve shells on a seasonal scale (Klein et al., 1996a; Lorrain et al., 2005; Gillikin et al., 2006, 2008; Wanamaker et al., 2008). Seasonal fluctuations of Sr/Ca and Mg/Ca ratios in bivalves are related to calcification temperature according to some studies (Richardson et al., 2004; Freitas et al., 2005, 2006; Wanamaker et al., 2008; Izumida et al., 2011; Yan et al., 2013). However, other authors advocate that these fluctuations rather reflect changes in metabolic rate and seawater salinity (Klein et al., 1996a; Steuber, 1999; Lorrain et al., 2005). Bivalve growth and metabolic rates are in turn driven by changes in external conditions, indicating that temperature and other seasonally
varying external parameters may ultimately control Mg/Ca and Sr/Ca ratios in some species (Gillikin et al., 2005; Tang et al., 2008; Schöne et al., 2013).

Because rudists have no close living relatives, there is no certainty as to the relationship between elemental ratios in their shells and environmental conditions. However, several studies show that elemental ratios measured at high resolution through rudist shells appear to record seasonal changes in environmental conditions (Steuber, 1999; Immenhauser et al., 2005). The novel μXRF method proposed here allows for fast, high-resolution, non-destructive analysis of elemental ratios in rudist shells. It rapidly collects the large quantities of data needed to shed light on the origin of elemental abundances in fossil bivalve shells and their application for palaeoenvironmental reconstruction. This study presents results of accuracy and repeatability tests of the μXRF method as well as a high-resolution μXRF profile across a shell of Vaccinites vesiculosus from the Late Campanian of Oman.

METHODOLOGY

Methods for trace element analysis

The approach proposed here uses the new generation Bruker® M4 Tornado table-top energy-dispersive μXRF scanner (Bruker Nano GmbH, Berlin, Germany; Fig. 1). X-ray fluorescence (XRF) allows the characterization of the elemental abundances in a sample from the fluorescence spectrum emitted after the sample is excited by an X-ray source (Norrish & Chappell, 1967). The μXRF technique combines the advantages of an automated microscope-guided high-precision movable stage system with the spectral resolution of a high-energy X-ray source to allow fast, non-destructive and high-resolution (25 μm) elemental analysis (Beckhoff et al., 2007; Gauglitz & Moore, 2014). To achieve the small spot size needed for high-resolution measurements, X-rays from either the rhodium or tungsten source are focused using a polycapillary lens (see Fig. 1). This focuses the X-ray beams with minimized energy loss, enabling the instrument to yield well-resolved XRF spectra of small surfaces on the sample without the use of a synchrotron radiation source. The polycapillary focusing and XYZ moving stage of the table-top μXRF device enable the formation of line scans and two-dimensional maps on the sample surface. The dimensions of the vacuum chamber of the μXRF device make it possible to measure sample surfaces of up to 200 mm × 160 mm. No sample coating is necessary and the method is fully non-destructive allowing, for example, the measurement of rare and unique samples from museum collections.

Fig. 1. Schematic overview of the Bruker M4 Tornado μXRF set-up showing the Rh X-Ray source (1), the poly-capillary optics (2), the Silicon Drift Detectors (3), the sample (4), the focused incident X-Ray beam (5), the X-ray computer-guided sample stage (6), the vacuum pump (7), the outgoing fluorescence hitting the detector (8) and the vacuum chamber (9). Note that the source (1) and optics (2) are positioned behind the field of view such that the incident beam (5) is aimed at an angle of 39° to the field of view. Besides their lateral angle shown in the picture, the detectors (3) are positioned to the front such that the outgoing fluorescence (8) hitting the detector forms at 51° with the field of view. As a result, the angle between incident (5) and outgoing (8) radiation is exactly 90°.
An excitation energy (30 W), which is 20 times higher than that of devices used in earlier studies (e.g. Kurunczi et al., 2001), and a vacuum chamber enable the generation of better-resolved point spectra and results in more accurate measurements in the lower energy part of the spectrum (<5 keV). Different source filters are included in the system to increase the spectral resolution for elements in the central energy range (5 to 15 keV) of the XRF spectrum. Source filters are thin (μm scale) films of metal placed in front of the X-ray source to block X-rays of certain energy ranges in order to reduce noise (and increase signal to noise ratio) in other regions of the spectrum (for example, 5 to 15 keV) where fluorescence peaks of elements of interest appear (Lee et al., 2001; Beckhoff et al., 2007). These improvements allow quantitative trace elemental analysis to be carried out rapidly in a non-destructive manner for all elements heavier than sodium.

**Acquisition of a Late Cretaceous rudist**

A fossil hippuritid rudist bivalve shell (*Vaccinites vesiculosus*; Woodward, 1855), originating from the Late Campanian of Saiwan area, Huqf Desert in east-central Oman [7 to 8 m stratigraphic height above Unit 1 of Schumann (1995), see also Kennedy et al., 2001; Platel et al., 1994], was obtained from the Oertijdmuseum in Boxtel (The Netherlands). Rudists in this formation are encountered in an upright position in large (kilometre-scale, Schumann, 1995) biostromes. The elongated right valve of the shell was cut longitudinally into a slab of ca 5 mm thickness using a diamond coated rock saw and polished using progressively higher grade silicon carbide polishing discs (up to P2400).

**μXRF scanning**

A Bruker® M4 μXRF scanner equipped with a Rh source and two XFlash 430 Silicon Drift detectors was used for obtaining the μXRF measurements (Fig. 1). The polished surface of the shell was mapped using short acquisition times per point (20 ms) for a qualitative assessment of the nature of the material. Line scan measurements were taken in growth direction (away from the umbo) on the prismatic outer layer of the shell. This part of the shell was least influenced by diagenesis, recognized by an absence of Mn and Fe intensity indicating calcite with minimal recrystallization (Brand & Veizer, 1980; Al-Aasm & Veizer, 1986; Ullmann & Korte, 2015; Fig. 2B). All line scan measurements were executed under vacuum conditions (20 mbar), with a 25 μm spot size and with a 3 second dwell time per point. Multiple line scans were combined into segmented lines to accommodate the curve in the growth axis of the shell (see Fig. 2A). After being automatically set up, the scan of the total shell length took ca 6 hours of measurement time. Every line scan was measured in duplicate: once without the source filter and with maximized source energy settings (600 μA, 50 kV), and once with maximized source current (800 μA, 35 kV) using the Al–Ti–Cu source filter (100 μm Al, 50 μm Ti and 25 μm Cu). Filter settings were selected after comparing XRF spectra generated using various filter settings. The filter was applied to obtain better peaks in the 5 to 15 keV domain of the XRF spectrum.

**Data analysis and repeatability**

Standard deviations of deconvolution (deconvolution errors) of XRF spectra were calculated using the Bruker ARTAX software on the sum-spectrum of each line scan. Results of individual points were accepted or rejected based on a detection limit of three standard deviations of deconvolution calculated by ARTAX (α ≈ 0.003; as in Rousseau, 2001). A quantification limit of 10 standard deviations of deconvolution (as proposed in Keith et al., 1983) was calculated for each element. Line scans were quantified by Bruker Esprit spectral processing software customized for the Bruker M4 using fundamental parameters quantification (Beckhoff et al., 2007). The attenuation length, a measure of penetration depth of X-rays into a material, is dependent on the mass absorption coefficient, density of the material and the energy of the X-ray photons (Bouguer, 1729). This means that different elements will have different attenuation lengths, and that in general measurements of heavier elements will average concentration over a larger volume in the sample (Sunjić & Šokčević, 1974; Potts et al., 1997). Using the Beer-Lambert law (Bouguer, 1729), it can be determined that the attenuation depth for carbonate samples in the energy range of XRF peaks used in this study (1 to 20 keV) does not exceed 2 mm (see supplementary data). The sample thickness of 5 mm is therefore sufficient for the assumption of total X-ray attenuation ("infinitely thick samples")
made in the Fundamental Parameters quantification procedure used by the Esprit software. Abundances of light elements, such as carbon and oxygen, could not be measured but were instead calculated in the deconvolution process from known stoichiometric ratios in carbonates. The deconvolution method was calibrated for calcium carbonate using the BAS CRM 393 ISO certified carbonate standard from the Bureau of Analyzed Samples Limited (BAS, Middlesbrough, UK). Calibration factors were chosen for each element based on repeated measurements of the standard to allow conversion of ‘raw’, semi-quantitative elemental abundance data into repeatable, quantitative data. Accuracy of the μXRF measurements was defined by the difference between calibrated values obtained through μXRF scanning on three ISO certified standards (BAS CRM393 limestone, BAS CRM512 dolomite and BAS ECKM782-1 dolomite) and certified values of the standards. Instrument repeatability was tested by repeated point measurements on spots on the rudist shell, as well as on homogenized calcium carbonate powder and on the three standards. The standard deviations between 30 repeated measurements (reported as repeatability errors in Table 1) were used as a measure of the precision.

Line scan records were analysed for significant periodic behaviour using a Fast Fourier Transform (FFT) procedure with Monte Carlo noise estimation (Muller & MacDonald, 2002; Bice et al., 2012; see supplementary data R1) with a 95% confidence level to test spectral peak significance. This method is applied frequently in cyclostratigraphy to test whether visually observed periodic signals are present in a palaeoclimate data set with statistical significance (e.g. Batenburg et al., 2012; De Winter et al., 2014; Zeeden et al., 2014). Here, it is
Table 1. Overview of mean values, detection limits and relative repeatability and accuracy errors obtained by μXRF for all detected elements. Red cells indicate errors that exceed 10% of the measured value. Grey cells indicate measurements in which the mentioned elements were either not detected or no certified concentration was available for the element. Where element names are highlighted in colour, these are not measured with confidence either because their values are not repeatable (red) or because they were not detected in most of the standards (grey).

<table>
<thead>
<tr>
<th>Element</th>
<th>Mean value in line scan (μg g⁻¹)</th>
<th>Repeatability</th>
<th>Powder (one spot)</th>
<th>Powder (varying spots)</th>
<th>Error of deconvolution</th>
<th>Detection limit (μg g⁻¹; 3 SD)</th>
<th>Quantification limit (μg g⁻¹; 10 SD)</th>
<th>Accuracy</th>
<th>CRM393</th>
<th>CRM512</th>
<th>CRM782</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>5530</td>
<td>8.2%</td>
<td>3.4%</td>
<td>9.7%</td>
<td>4.0%</td>
<td>655</td>
<td>2184</td>
<td></td>
<td>n.d.</td>
<td>−7.4%</td>
<td>n.d.</td>
</tr>
<tr>
<td>Mg</td>
<td>4744</td>
<td>2.8%</td>
<td>0.9%</td>
<td>1.7%</td>
<td>2.7%</td>
<td>389</td>
<td>1298</td>
<td></td>
<td>n.d.</td>
<td>−0.1%</td>
<td>−0.3%</td>
</tr>
<tr>
<td>Al</td>
<td>1132</td>
<td>6.0%</td>
<td>7.9%</td>
<td>10.3%</td>
<td>6.0%</td>
<td>205</td>
<td>682</td>
<td></td>
<td>−0.5%</td>
<td>−2.1%</td>
<td>−2.1%</td>
</tr>
<tr>
<td>Si</td>
<td>4041</td>
<td>0.6%</td>
<td>0.5%</td>
<td>0.8%</td>
<td>0.3%</td>
<td>38</td>
<td>125</td>
<td></td>
<td>−0.6%</td>
<td>−0.1%</td>
<td>−0.8%</td>
</tr>
<tr>
<td>P</td>
<td>273</td>
<td>11.9%</td>
<td>10.2%</td>
<td>14.7%</td>
<td>1.7%</td>
<td>14</td>
<td>47</td>
<td>−13.7%</td>
<td>−35.9%</td>
<td>−5.8%</td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>13</td>
<td>16.2%</td>
<td>19.7%</td>
<td>23.1%</td>
<td>9.3%</td>
<td>3.7</td>
<td>12</td>
<td>−1.3%</td>
<td>2.8%</td>
<td>−0.6%</td>
<td></td>
</tr>
<tr>
<td>Cl</td>
<td>11 721</td>
<td>0.1%</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.0%</td>
<td>15</td>
<td>50</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Ca</td>
<td>383 224</td>
<td>0.1%</td>
<td>0.0%</td>
<td>0.1%</td>
<td>0.0%</td>
<td>310</td>
<td>1032</td>
<td>−0.3%</td>
<td>−1.4%</td>
<td>−31.9%</td>
<td></td>
</tr>
<tr>
<td>Ti</td>
<td>179</td>
<td>1.3%</td>
<td>n.d.</td>
<td>4.2%</td>
<td>1.6%</td>
<td>8.3</td>
<td>28</td>
<td>−0.3%</td>
<td>−1.7%</td>
<td>1.1%</td>
<td></td>
</tr>
<tr>
<td>Cr</td>
<td>55</td>
<td>0.3%</td>
<td>0.3%</td>
<td>0.9%</td>
<td>0.1%</td>
<td>0.2</td>
<td>0.8</td>
<td>n.d.</td>
<td>−3.3%</td>
<td>−9.9%</td>
<td></td>
</tr>
<tr>
<td>Mn</td>
<td>303</td>
<td>0.7%</td>
<td>0.7%</td>
<td>3.9%</td>
<td>0.7%</td>
<td>6.3</td>
<td>21</td>
<td>−1.0%</td>
<td>0.1%</td>
<td>1.2%</td>
<td></td>
</tr>
<tr>
<td>Fe</td>
<td>2187</td>
<td>0.2%</td>
<td>0.1%</td>
<td>0.6%</td>
<td>0.1%</td>
<td>6.6</td>
<td>21</td>
<td>−0.4%</td>
<td>0.3%</td>
<td>0.7%</td>
<td></td>
</tr>
<tr>
<td>Ni</td>
<td>58</td>
<td>16.8%</td>
<td>n.d.</td>
<td>6.3%</td>
<td>0.1%</td>
<td>0.3</td>
<td>0.8</td>
<td>n.d.</td>
<td>2.4%</td>
<td>2.1%</td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>27</td>
<td>33.5%</td>
<td>27.7%</td>
<td>47.5%</td>
<td>5.5%</td>
<td>4.4</td>
<td>15</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>167</td>
<td>0.3%</td>
<td>0.4%</td>
<td>2.0%</td>
<td>0.2%</td>
<td>1.0</td>
<td>3.5</td>
<td>n.d.</td>
<td>0.1%</td>
<td>−5.8%</td>
<td></td>
</tr>
<tr>
<td>Sr</td>
<td>416</td>
<td>1.0%</td>
<td>0.6%</td>
<td>5.1%</td>
<td>1.0%</td>
<td>13</td>
<td>42</td>
<td>1.1%</td>
<td>0.2%</td>
<td>n.d.</td>
<td></td>
</tr>
<tr>
<td>Pb</td>
<td>44</td>
<td>8.6%</td>
<td>10.5%</td>
<td>15.3%</td>
<td>1.0%</td>
<td>1.4</td>
<td>4.5</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>477 307</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>118 345</td>
<td>0.1%</td>
<td>0.0%</td>
<td>0.1%</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td></td>
</tr>
</tbody>
</table>
applied on μXRF data in an attempt to detect seasonal cyclicity in the records.

**Cathodoluminescence**

A thick section of the above-mentioned rudist specimen was examined under a Zeiss Universal-R microscope (Carl Zeiss Microscopy GmbH, Oberkochen, Germany) adapted for cathodoluminescence (CL) with a CITL Mk5 cold cathodoluminescence unit (Cambridge Image Technology Limited, Welwyn Garden City, UK), available at the Department of fundamental and applied geology at Université de Mons, Belgium. All CL images were taken at 50× magnification using an excitation energy of 15 kV and 500 μA, and an exposure time of 48 ms.

**RESULTS**

**μXRF spectra**

Figure 3 displays examples of μXRF sum spectra of line scans used for quantification. The figure shows that the spectra obtained by the μXRF line scans display distinct peaks for all indicated elements. Except for Na, the heights of all peaks are bigger than the noise observed on the background of the spectrum. All elements shown in Fig. 3 are detected by the Esprit software. Signal to noise ratios of peaks in the lower energy range, such as Na, Mg and Al, are lower (<2.0, see Fig. 4), causing quantified concentrations of these elements to be less repeatable, as is evident from the relatively high measurement errors reported for these elements (Table 1). Figure 3 also shows the difference between spectra created with and without a source filter. In the energy range of ca 5 to 15 keV, element peaks are better resolved (higher signal to noise ratio) in the spectrum made using the source filter. The count rate (counts per sec) is lower in the filtered spectrum compared to the unfiltered spectrum. At the same time, peaks in the low energy range (such as Na and Mg) have a better (higher) signal to noise ratio in spectra obtained without source filter. This demonstrates the benefit of combining measurements with and without filters. Based on these results, all line scans are taken in duplicate and elements in the 5 to 15 keV range are measured using the Al–Ti–Cu filter in this study. Deconvolution errors, detection limits and quantification limits calculated from XRF spectra are reported in Table 1.

**Repeatability of μXRF results**

Table 1 gives an overview of the repeatability errors calculated from variation between

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**Fig. 3.** Graph showing sum spectra of the middle segment of the composite line scan taken without (A) and with (B) the Al–Ti–Cu filter. The grey area marks the difference between the spectra. Note that the vertical scale is in counts per second per energy increment and does not represent the area underneath elemental peaks.

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successive measurements on the same spot on the rudist bivalve and on homogenized calcium carbonate powder. Repeatability errors of measurements on different spots of varied location on the surface of the homogenized carbonate powder are also reported. Repeatability is highly variable between different elements and ranges from 0-1 to 10-0%. Repeatability errors are similar for a spot on the bivalve shell and a spot on powdered carbonate. The repeatability standard deviations of different spots on the homogenized carbonate powder range from 0-1 to 15-0% for most elements and are slightly higher than the single-spot repeatability error. This indicates that surface effects caused by slight changes in X-ray scattering due to varying surface roughness introduce error in μXRF measurements, even on well-homogenized standards. The repeatability error calculated from multiple points on the standards is always higher than the deconvolution error calculated from the spectrum, indicating that this spectral deconvolution error underestimates the total error of μXRF measurements, and that repeatability tests are always needed to assess the full analytical error of these measurements.

Results in Table 1 show that all standard deviations are higher for lighter elements (Na and Mg) and for elements situated in the energy range for which a filter is used (5 to 15 keV: elements Ti to Cu). Repeatability for these elements (especially Na and Mg) decreases along with the error of deconvolution. Both deconvolution and repeatability errors are higher for elements that are present in lower concentration (for example, P, S and Cu, see Table 1). There is no straightforward relationship between mean concentration and standard deviation, because some elements with a relatively low concentration (for example, Ti and Sr) have comparatively low errors. This shows that elements in certain energy ranges (for example, in the middle of the spectrum) are more easily detected and quantified by Rh-source μXRF scanning than other elements; a feature that is reflected in the variation of signal to noise ratios of element peaks within a μXRF spectra (see Fig. 4).

Accuracy of μXRF measurements

After calibration with the BAS CRM 393 standard, the resulting elemental abundances of point measurements on the same spot on
measurements on the standard are within 1.5% (ca 5 µg g⁻¹ for Sr) of the given value for all certified elements except P for the CRM393 standard (Table 1). For the dolomite standards, accuracies are in the same order of magnitude and P concentrations are also less accurate. In addition, offsets in CRM782 are also high (>5%) for Ca, Cr and Zn. The offset between measured values and certified values is lower than the repeatability error for all other elements. The concentration of P in the standards (14 µg g⁻¹, 53 µg g⁻¹ and 67 µg g⁻¹ for CRM393, CRM512 and ECRM782, respectively) is lower than the mean concentration in the line scan (273 µg g⁻¹). It is therefore likely that the P concentration in the standard is too low to quantify using μXRF. Indeed, Table 1 shows that the repeatability errors of P are already relatively high (>10%) for the higher P concentrations in the V. vesiculosus shell and the concentration of P in the standards approaches or even exceeds the quantification limit (47 µg g⁻¹). High offsets in more common elements that otherwise show good reproducibility (for example, Ca and Zn) are most likely to be a result of the difference in matrix between the dolomite (CRM512 and ECRM782) and calcite (CRM393) standards.

Mapping and facies characterization

Results from μXRF mapping of the entire polished shell surface (Fig. 2B) reflect the internal structure of the rudist shell. The short acquisition time for map pixels (20 ms) causes the detection and quantification limit for trace elements to be much higher than in point measurements or line scans. Map results as shown in Fig. 2B are therefore only suitable for qualitative assessment of the nature of the sample material. Traces of Mn, Si and Fe that show up on the map in Fig. 2B are present in relatively high abundance and are probably signs of diageneric alteration of the shell rather than primary traces incorporated during shell growth. The inside of the shell consists of porous calcite with local enrichments in Mn, Si and Fe, while the outer shell layer shows lower concentrations of these elements and higher Ca and Sr values indicative of more pristine calcite. This shows that the outer shell layer is more suitable for analysis of biogenic trace element profiles and successive palaeoenvironmental reconstruction. Within the outer shell layer, spots of two types of enrichments are visible: more common enrichments with Mn, Fe and Si (magenta) and less frequent enrichments of Mn alone (yellow). The Mn–Fe–Si enrichments are observed in spots throughout the outer shell layer with a relatively high density in the lower and upper part of the shell (near the beginning and end of the line scan). The Mn enrichments are observed in the inner part of the shell, but a few bright yellow Mn-spots occur in the outer shell layer. Cathodoluminescence pictures of some parts of the outer shell layer calcite show that the Mn–Fe–Si enrichments have a dull luminescence and are rounded in shape with a detrital inner texture (Fig. 2C-II, 2C-IV and 2C-VI). Some have centres of more crystalline content that show bright luminescence (Fig. 2C-II, top). These enrichments are brown in the colour scan shown in Fig. 2A. The Mn enrichments are larger and filled with a crystalline texture which has very bright luminescence (Fig. 2C-V). These enrichments are white in Fig. 2A. The microscope images reveal that both enrichments cut through the surrounding calcite, which luminesces weakly and shows well-preserved internal laminations (Fig. 2C-I, 2C-II and 2C-V). Internal laminations of the well-preserved calcite facies in the outer shell layer sometimes exhibit bands of brighter luminescence (Fig. 2C-III). These bands follow the original internal lamination of the calcite and appear to be primary in nature.

Elemental abundances in Vaccinites vesiculosus

Combined μXRF line scans on the V. vesiculosus shell yielded an 86-9 mm long record of the abundance of 18 elements, consisting of 3503 point measurements with an average spatial resolution of 24-82 µm (see Fig. 5, data of all individual points are reported in supplementary data). While average elemental abundances from line scan measurements on the V. vesiculosus shell exceed the detection limits (Table 1), individual points on the line yield concentrations below this limit, especially for Na, Mg and some of the elements with peaks in the 5 to 15 keV domain (see Fig. 3). Quantification limits are highly variable between elements, and high detection limits cause most of the values found for some elements (for example, Na and Mg) to be rejected, leading to gaps in the record (see Fig. 7A and supplementary data). Therefore, profiles of Na and Mg cannot be measured quantitatively in bivalve calcite using the set-up and measurement strategy of this study.

The count rate in the Ca peak shows sharp downward excursions in some parts of the
These drops in XRF counts are associated with fluctuations in (otherwise rather constant) Ca concentrations. Both effects are indicative of irregularities in the sample surface or changes in Ca content in the sample. These parts are highlighted in Fig. 5.

Based on their concentrations given in Table 1, elements in the record can be divided into major elements (Ca, O and C) with average concentrations above 1% and trace elements with average concentrations in the per mille (mg g\(^{-1}\)) to ppm (µg g\(^{-1}\)) range (all other elements). Concentrations below 1 µg g\(^{-1}\) were generally below the detection limit, demonstrating the lower limits of detection and quantification of the µXRF method and set-up used in this study (Table 1). Table 1 shows that Ca concentrations are around 38% by weight (ca 380 000 µg g\(^{-1}\)), as expected for calcite samples. Most point measurements on the line scan were representative of bivalve calcite, but at some points trace element concentrations (for example, Si, Mn and Fe) are higher and Ca and Sr concentrations lower, suggesting detrital inclusions (Fig. 5, indicated in red). The presence of such inclusions and recrystallization is also visible on the XRF mapping and cathodoluminescence images shown in Fig. 2C. Many other elements have ranges that include very low values, indicating point measurements in which the element was not detected or that the concentration of the element was below the detection limit of 3σ (Table 1). Some elements (for example, Cl, Mg, Zn and Cr) have a high range of variability compared to their mean concentrations, indicating either that many measurements of these element concentrations fall below the confidence level or that the elements are highly variable in the bivalve record. Except for Mg, repeatability errors for these elements (Table 1) are relatively low, favouring the latter explanation.
Intra-shell trace element records

To give a quick overview of the relative variation in quantified elemental abundances along the line of measurement in the growth direction of the shell, a normalized Heatmap is made using the R statistics package (Fig. 6A). A Heatmap is a qualitative representation of the extensive data matrix obtained by μXRF scanning, and is obtained by normalizing every elemental record and plotting the normalized variation in the records against distance along the shell by means of a colour scale (see Wilkinson & Friendly, 2009 and appendix R2). The Heatmap (Fig. 6A) shows the variation in all measured elements at a glance and allows direct qualitative comparison between the records of different elements while avoiding the cumbersome plotting of all 18 elemental abundance records in one figure.

A significant portion of the variation in multi-proxy record is explained by variation in Ca content, which is indicative of the success of the measurement and the purity of the calcium carbonate sample. To illustrate changes in trace element records regardless of this Ca variation, Fig. 6B shows a Heatmap of elemental abundances relative to Ca. This Heatmap of elemental ratios shows that Mg/Ca and Si/Ca seem to covary in the *V. vesiculosus* record. Figure 6B also shows a strong covariation of Mn/Ca, Fe/Ca and Sr/Ca records. A detailed plot of these records against shell length (Fig. 5) shows that records of Si/Ca, Mn/Ca and Fe/Ca have a lower range of values (<5000 µg g⁻¹, <400 µg g⁻¹ and <3000 µg g⁻¹, respectively). Sharp excursions towards higher values protrude from this range of low values. Some of the larger peaks in Mn/Ca and Fe/Ca (exceeding 1200 µg g⁻¹ and 10 000 µg g⁻¹, respectively) coincide with maxima of Si/Ca (exceeding 10 000 µg g⁻¹), sharp drops in Sr/Ca (<600 µg g⁻¹) and minima in count rate. These shifts occur roughly every 8 mm in the upper and lower part of the record, and are less common in the interval between 30 mm and 70 mm from the umbo. Values in the Sr/Ca record vary between 800 µg g⁻¹ and 1500 µg g⁻¹ in parts of the records unaffected by the above-mentioned aberrations. In parts of the line scan not marked by peaks in Fe/Ca, Mn/Ca and Si/Ca, the Sr/Ca record seems to follow a periodic pattern with a period of ca 20 mm. Error bars in Fig. 5 based on two standard deviations of reproducibility tests on the CRM393 standard (see Table 1) show that the observed amplitude in all plotted records is statistically significant. The covariation of Fe/Ca, Mn/Ca and Si/Ca records suggests a similar forcing of variations in all four records with an opposite effect on Sr/Ca compared with the other three

![Fig. 6. Heatmap showing the normalized variation in all elemental records measured in the line scan (A) and in records of all elements divided by Ca (B). Red colours represent higher values and blue colours represent lower values. Vertical scale represents the distance from the umbo of the *Vaccinites vesiculosus* shell.](image-url)
Spectral analysis

A third normalized Heatmap illustrates the FFT periodograms for all elemental records (Fig. 7) and shows at a glance which records exhibit cyclical variation and whether the same periodicity is found in different records. The Heatmap highlights hotspots of significant periodicity in the above-mentioned Mn, Fe, Si and Sr records at 8 to 9 mm as well as around 20 mm in Sr and Ca records. Plots of corresponding FFT powerspectra for these elemental records (Fig. 8) illustrate the statistical confidence (‘power’) of cyclic variation in these records and where these exceed the 95% confidence level (red line). Figure 8 shows that peaks around 8 to 9 mm and 20 mm are indeed significant above a 95% confidence level in the records in which these periodicities were observed. Other periods seem to exceed the confidence level according to Fig. 8, but these are not observed in the µXRF data (Fig. 5). Figure 5 shows that variations in Si, Mn and Fe in the record are spaced more or less 8 mm apart and explain the periodicity. The larger 20 mm periodicity is visible in the Sr/Ca record in places on the records where peaks in Mn, Fe and Si are absent.

DISCUSSION

Method comparison

Results show that the repeatability of the µXRF used in this study (1% for Sr/Ca, see Table 1) competes with that of other techniques. In comparison, inductively coupled plasma – mass spectrometry (ICP-MS) that requires more sample preparation, yields accuracies within 3% and a repeatability of ±3% for elemental ratios in bivalve calcite (Lorrain et al., 2005), while destructive sampling is required for this method. In situ laser ablation – ICP-MS (LA–ICP–MS; e.g. Lazareth et al., 2003; Gillikin et al., 2005, 2008; Carré et al., 2006; Yan et al., 2013) achieves high spatial resolution (up to 50 µm), is faster and less destructive (although not entirely non-destructive), but it is more expensive than conventional ICP-MS and less easily accessible. This method yields accuracies within 10% (up to 4%) and repeatabilities of ≤5% on bivalve material (Vander Putten et al., 2000; Gillikin et al., 2005). Other methods are inductively coupled plasma – optical emission spectroscopy (ICP–OES; Yan et al., 2013; Sørensen et al., 2015) and atomic emission spectroscopy (ICP–AES; Klein et al., 1996a,b; Steuber, 1999). Both methods yield analytical precisions between 5% (Freitas et al., 2009) and 2% (Klein et al., 1996b; Ullmann et al., 2013; Yan et al., 2013). These methods, like conventional ICP–MS, require sample drilling and digestion of 0.5 to 1.0 mg of carbonate and are therefore destructive and rather time-consuming; a disadvantage in palaeoenvironmental studies that often require large data sets to yield comprehensive results.

Conventional XRF measurements require destructive sampling and the production of pellets to overcome the matrix effect on XRF measurements (Rowe et al., 2012). Real non-destructive and high-resolution (down to 1 µm) XRF measurements can be achieved by synchrotron µXRF (Thorn et al., 1995; Frisia et al., 2005; Jones et al., 2009; Lerouge et al., 2010).

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The X-ray beam is focused on a small spot on the sample to allow XRF measurements to be executed on a small (micro) spatial scale. While this method yields high precision (<5%) and accuracy (better than ±2 μg g⁻¹ for Sr; Thorn et al., 1995), it requires access to a very complex and costly synchrotron set-up where access and beam–time have to be requested, often through competitive proposal writing. At the moment, only 47 Synchrotron Light Sources are available worldwide (www.lightsources.org) and they are under heavy demand by the scientific community in a large variety of domains (e.g. Yoshimura et al., 2013, 2014; Nguyen et al., 2014).

Results of μXRF line scanning on a fossil Vaccinites vesiculosus shell show that the Bruker M4 Tornado μXRF scanner is capable of producing well-resolved XRF spectra with a ca 25 μm spot size resolution (Fig. 3). Compared with earlier studies with previous table-top capillary-source μXRF analysers (e.g. Kurunczi et al., 2001), the spectra obtained in the present study better resolve element peaks while spot size has decreased from 100 to 25 μm. Spectral resolution (signal to noise ratio) of the μXRF results is comparable to that of quantifiable spectra generated by synchrotron μXRF (e.g. Fria et al., 2005; Jones et al., 2009; Lerouge et al., 2010). Spectrum deconvolution software used in this study shows low detection limits (13 μg g⁻¹ for Sr), reduced standard deviations and a better resolved spectrum compared to Kurunczi et al. (2001). The selective use of the Al–Ti–Cu filter further increases the signal to noise ratio of peaks in the range of 5 to 15 keV (see Fig. 4).

The accuracy error of 1·1% (one standard deviation) relative to the measured value (ca 5 μg g⁻¹ for Sr concentration, see Table 1) is only slightly higher than that obtained by synchrotron analysis (±2 μg g⁻¹ Sr; Thorn et al., 2010).
1995) and slightly better than that obtained by ICP-MS (3% of the standard value; Lorrain et al., 2005). The measurement error (1 standard deviation) found in this study is 5% for Sr and better for most other quantifiable trace elements (Table 1), indicating that repeatability of μXRF in this study is similar to or better than the precisions reported for other methods (Thorn et al., 1995; Klein et al., 1996b; Lorrain et al., 2005; Yan et al., 2013). Conservative detection (3σ) and quantification (10σ) limits calculated from deconvolution errors show that the Bruker M4 μXRF is capable of reliably quantifying concentrations of most commonly studied elements down to the (tens of) μg g⁻¹ level, although quantification limits quickly increase for lighter elements.

The large spread in measured concentration observed for some elements indicates that the concentrations of elements like P, Cr, Cu, Zn and Pb in bivalve calcite are close to the limit of detection of μXRF (Table 1). This leads to a decrease in the accuracy of the measurement and causes more measurements to be rejected for being below the 3σ detection limit. The records of Si, Mn, Fe and Sr have comparatively low errors and concentrations that are well above their quantification limits (see Table 1). This indicates that measurements of these elements are more repeatable and have the best potential for recording changes in the composition of the rudist shell calcite. Records of elements in which many points are rejected are less suitable for this purpose.

Surface effects on repeatability

Differences between the repeatability error of reiterated measurements at the same spot and at different spots on the surface of powdered samples and variations in count rates of Ca in carbonate measurements indicate that the precision error of the μXRF technique varies with location on the sample surface. Surface-specific errors can be caused by so-called sample matrix effects that influence the backscattering of X-rays (Rousseau & Boivin, 1998; Beckhoff, et al. 2007). Such matrix effects cause accuracies on the dolomite standards (CRM512 and ECRM782) to be different from those on the limestone standard (CRM393). Variations in sample matrix should be fairly low within a homogenized carbonate sample, but small differences on the micrometre scale can still produce small measurement errors. A large part of this uncertainty is reduced by calibrating the peak deconvolution procedure using a standard with a matrix as similar to that of the sample as possible (Rousseau, 2001), as has been done in the present study. For this reason, changes in the sample matrix, such as the incorporation of detrital inclusions or recrystallized parts of the rudist shell, can offset measurement results because the measured spot does not match the matrix of the standard used to calibrate the method. For optimal results, μXRF line scans and maps must be done on samples with the same (or similar) matrix.

Another source for errors on the spot measurements is the roughness of the surface. Rough surfaces and local features such as cracks and depressions scatter X-rays and cause the beam to be out of focus when hitting the sample surface (Beckhoff et al., 2007). This effect explains a drop in count rate and unrealistic Ca values around 52 mm from the umbo of the rudist shell. From Fig. 2, it is clear that this drop in count rate is caused by a large crack in the sample where two parts of the rudist shell were reattached after sawing. This shows that polishing of sample surfaces to avoid effects of surface roughness will yield better results for line scans or maps. Rough surfaces can still be measured but will yield less repeatable results. Count rates of Ca in carbonate samples should be consistently high, and lower count rates in the Ca energy band are indicative of scattering of X-rays due to irregularities on the sample surface or changes in sample matrix and should therefore be treated with caution. The comparison between same-spot measurement errors (equivalent to polished surfaces) and different-spot measurement errors (equivalent to unpolished surfaces; Table 1) shows that measurements on a rough surface could be quantified and interpreted if the additional error of surface roughness is taken into account. This approach enables the measurements of unpolished surfaces of precious samples and shows that the μXRF method remains essentially non-destructive.

Detection of diagenesis

High concentrations of Mn and Fe in fossil bivalve shells have been proposed to reflect diagenetic alteration (Brand & Veizer, 1980; Veizer, 1983; Al-Aasm & Veizer, 1986; Ullmann & Korte, 2015). Previous studies have therefore tested against high concentrations of Mn and Fe in
rudist calcite and rejected samples in which concentrations were deemed too high to be pristine (>20 μg g⁻¹ and >200 μg g⁻¹ for Mn and Fe, respectively; Steuber et al., 2002). The highest values in the Mn/Ca and Fe/Ca records surpass these conservative thresholds, suggesting that diagenetic alteration is present in this rudist shell. However, results of studies recommending these threshold values fail to show with confidence that samples with higher Mn and Fe concentrations are necessarily diagenetically altered, and some studies have even shown that pristine Mn/Ca ratios can exceed thresholds used to assess whether diagenesis has occurred (Veizer, 1983; Steuber, 1999; Steuber et al., 2002; Ullmann & Korte, 2015; and references therein), rendering the distinction of diagenetic trace element enrichment from primary incorporation difficult based on trace element profiles alone.

Cathodoluminescence microscopy together with XRF mapping (Fig. 2B and C) shows that the majority of the outer shell layer consists of pristine calcite characterized by well-preserved growth structures. Concentrations of elements in parts of the line scan from these pristine areas are in agreement with values reported in other studies of fossil bivalve calcite (Steuber, 1999; Steuber et al., 2002) and modern bivalve calcite (Gillikin et al., 2005; Lorrain et al., 2005; Jacob et al., 2008). The high mean concentration of Ca (38.3%, see Table 1) in the line scan shows that the majority of the line scan points are from pristine calcite (Fig. 5). The high abundance of Si, Mn and Fe in parts of the record indicates that some parts of the shell contain siliciclastic particles. The presence of Si in bivalves indicates either contamination with siliciclastic material into the shell structure or alteration of the shell after burial (Hendry et al., 1995; Klein & Walter, 1995; Elorz & García-Garmilla, 1996). Calcite formation in bivalves typically occurs inside the extrapallial space of the animal, separated from direct seawater influence (Carré et al., 2006; Jacob et al., 2008). The incorporation of detrital matter into the shell structure of rudists can nonetheless occur due to rupture of the periostracal rim (Skelton et al., 1990) and/or boring of epifaunal organisms (Lazo, 2004). The Heatmaps show that high values in Si/Ca ratio sometimes coincide with those in Fe/Ca and Mn/Ca (Fig. 6B). The CL images in Fig. 2C show that these enrichments in Si are linked to the rounded features that exhibit dull luminescence. These features are also observed in the XRF mapping of the shell surface and are more common near the lower part of the shell. Spikes in Si, Mn and Fe ratios in the records (Fig. 5) are caused by the scan line passing over one of the detrital inclusions. The round shape of most of the detrital inclusions observed in CL suggests that they are caused by borings of molluscs or other epifaunal organisms (Fig. 2; Sanders & Pons, 1999). The fact that most of the inclusions occur on the outside of the calcite layer (see Fig. 2C-IV) supports this assumption. The CL images in Fig. 2C show that these borings are easily observed in the XRF mapping image (Fig. 2B) because their dimensions surpass the spot size of the μXRF. This shows that XRF mapping can be a valuable tool in recognizing and localizing diagenetic alteration in rudist shells.

Recrystallization in the shell is associated with an increase in Mn, while Fe and Si remain low. Some peaks in Mn in the record, which are not coinciding with peaks in Fe and Si, could therefore be interpreted as recrystallization. This recrystallized calcite is most likely to be a result of sparite filling in pores that form when inclusions into the shell dissolve, because recrystallization of the original low-Mg calcite of the outer shell is unlikely to have happened only locally (Al-Aasm & Veizer, 1986; Ullmann & Korte, 2015). An example of this is shown in Fig. 2B-II where part of the detrital cavity is later filled in with brightly luminescing sparite. However, cathodoluminescence images (for example, Fig. 2C-III and 2C-V) also show that bright luminescence can occur in certain growth layers in the rudist shell, indicating that some increases in Mn can have a primary origin. The bright luminescence on the inside of the outer shell layer shows that the inner shell layers, which are aragonitic in Hippuritidae (Skelton, 1976; Al-Aasm & Veizer, 1986), have completely recrystallized. The difference between recrystallized parts of the shell and the enrichments of Mn in the growth layers of pristine calcite is easily spotted in the XRF map of Fig. 2B. The large difference in Mn concentration between the recrystallized parts and the primary Mn incorporation results in a large difference in brightness of the Mn mapped by the μXRF scanner.

Strontium/Calcium record and seasonality

In parts of the record that are unaffected by detrital inclusions, variations in the Sr/Ca
record are of the same order of magnitude as those reported in other studies of seasonal Sr/Ca fluctuations (e.g. Richardson et al., 2004; Freitas et al., 2006; Yan et al., 2013). Absolute Sr/Ca values resemble those found in other rudist studies (e.g. Steuber, 1999). The general trend in the Sr/Ca record seems sinusoidal with a 20 mm cycle and may exhibit annual forcing. The 8 mm cycle is most likely to be a result of the spacing of the detrital inclusions. If these inclusions are indeed borings, no primary palaeoenvironmental interpretation can be attributed to this 8 mm cycle. The 20 mm cycle observed in Sr/Ca ratios may be a result of annual retreats of the periostracal rim, allowing more trace elements to enter the shell structure and creating bands of calcite enriched in trace elements (Skelton et al., 1990). If a growth rate of ca 20 mm per year is assumed, four to five years of growth may be present in this rudist shell. Records with periodicity on a similar spatial scale have been interpreted as seasonal cyclicity in studies of rudist bivalves, and the resulting growth rate of 20 mm year\(^{-1}\) is not unusual for rudist bivalves (Steuber, 1999; Steuber et al., 2005).

Although used as palaeothermometer in many bivalve studies, the temperature dependence of Sr/Ca in rudists is controversial, due to changing Sr/Ca ratios of seawater and the lack of a well-established correlation of Sr/Ca with \(^{8}\)Sr/Ca records (Opdyke et al., 1995; Steuber & Veizer, 2002; Dutton et al., 2007). Due to this lack of correlation and the possibility that detrital inclusions caused Sr concentrations to decrease, no attempt is made here to use variations in Sr/Ca of V. vesiculosus to quantify seasonal changes in temperature.

Besides temperature, metabolic and calcification rates have also been proposed as drivers for seasonal variations in Sr/Ca in bivalve calcite, due to their effect on the equilibrium between Sr/Ca values of seawater and Sr/Ca values in the extrapallial fluid from which bivalves crystallize their calcite (Klein et al., 1996b; Gillikin et al., 2005; Lorrain et al., 2005). If these factors drive the Sr/Ca variations observed in this study, minima in Sr/Ca that are not caused by detrital contamination may correspond to increases in metabolism and decreases in skeletal growth rate (Klein et al., 1996b), which in turn may reflect the seasonal reproductive cycle as observed in modern bivalves (Pichaud et al., 2009). It has been suggested that metabolic rates in bivalves are directly related to food availability and temperature, which are highly seasonal parameters (Blicher et al., 2010). All of these elements point towards a seasonal forcing of Sr/Ca ratios in bivalve calcite. Following this rationale, Steuber (1999) proposed that shifts in Mg and Sr observed in Vaccinites rudist shells might be controlled by metabolic rate. Increasing metabolic rate and decreasing shell growth rate are expected in spring, when food availability is high and metabolic rate increases (Blicher et al., 2010). This pattern also matches the reproduction cycle with spawning of offspring in spring (Steuber, 1999; Pichaud et al., 2009). Such a fast metabolic rate in spring together with a spawning event might create the conditions in which a deep withdrawal of mantle lobes and/or a tearing of the periostracal rim facilitates the incorporation of external trace elements into the shell structure, explaining the observed increases in brightness in CL of some growth layers observed in Fig. 2C-I and 2C-III (Skelton et al., 1990; Lawfield, 2014). This can in part explain the correlation on the 20 mm scale that is observed between Sr/Ca on the one hand and Mn/Ca, Fe/Ca and Si/Ca on the other hand.

**Other trace element records**

Small Mn/Ca variations that are not associated with detrital inclusions observed in the record of V. vesiculosus express cyclic variations similar to those observed in modern bivalves, where these fluctuations are associated with seasonal variations in primary productivity (Freitas et al., 2006). The increases of Mn/Ca observed in V. vesiculosus that are not complemented by similar increases in Fe/Ca and Si/Ca (see Fig. 5) are paced to the above-mentioned fluctuations observed in Sr/Ca and are always in the opposite direction. Smaller fluctuations in Fe/Ca (<1000 \(\mu g \cdot g\)^{-1}) in bivalve calcite have been related to an increase in riverine discharge and changing redox conditions, (Bruland et al., 2001; Gao et al., 2009). The formation of spring blooms in the Late Campanian Tethys Ocean could provide such changing redox conditions, and could explain small peaks in Mn/Ca and Fe/Ca observed in V. vesiculosus wherever these are not matched in the Si/Ca record and where Fe/Ca peaks do not exceed levels that are considered likely in primary calcite (<1000 \(\mu g \cdot g\)^{-1}; Steuber, 1999; Brand et al., 2003). Spring bloom formation is postulated to be a significant driver of changes in trace element concentrations in bivalves (Lazareth et al., 2003). Therefore, non-diagenetic peaks in Mn and Fe ratios coinciding
with minima in Sr/Ca may be signs of increased palaeoproductivity during spring blooms in the Late Campanian. Such a relationship would imply that shell Sr/Ca ratios were low in the relatively cool spring season, which would be in agreement with a positive relationship between Sr/Ca and temperature found in modern bivalves (e.g. Richardson et al., 2004). It is therefore possible that changes in temperature and metabolic rate influence the Sr/Ca ratio in this rudist bivalve on a seasonal scale. However, temperature seasonality in the Late Campanian low-latitude Tethys Ocean may not have been large enough to cause a significant effect on the Sr/Ca ratios in bivalve calcite. Because detrital incorporation is interfering with the Mn and Fe records in this rudist, other rudist specimens need to be examined to study the relationship of these trace elements and their value in palaeoenvironmental reconstruction. Comparison with other proxies, such as stable isotope ratios, may shed more light on the exact origins of seasonal cycles observed in rudists.

CONCLUSIONS

New developments in the technology of tabletop tube excitation micro X-ray fluorescence (μXRF) scanners enable the rapid and precise detection and quantification of concentrations of elements in fossil rudist bivalves, with errors of accuracy and precision that compete with other more expensive, destructive and time-consuming methods. This non-destructive method makes it possible to analyse precious or rare materials such as museum specimens. Advanced peak deconvolution software together with high measurement energies (30 W) and application of source filters in the Bruker M4 μXRF device creates well-resolved XRF spectra with high signal to noise ratios using relatively short (3 sec) measurement times per point. This approach makes quantifiable high-resolution line scanning of sample surfaces possible within a realistic timeframe and allows variations in major as well as trace elements to be measured with confidence. However, the detection limit is highly variable between elements, causing the error on quantified values of some elements (for example, Na and Mg) measured in this study to be too high for intra-shell comparison. A conservative quantification limit must be taken into account when deciding which elements can be quantified reliably.

The use of μXRF trace element mapping combined with cathodoluminescence microscopy is an efficient way to detect diagenesis in fossil bivalve shells and to interpret trace element profiles measured with μXRF. This combination shows that μXRF mapping can be used to detect recrystallization as well as detrital input into the calcitic outer shell layer of the rudist bivalve. Application of a Fast Fourier Transform spectral analysis routine with noise simulation allows assessment of the significance of cyclicity in elemental records of this rudist bivalve. It is applied successfully here to detect seasonal changes in these records. After detection and removal of the parts of the record that were altered by diagenesis, records of Mn and Fe in Vaccinites vesiculosus show increases that may reflect seasonal augmentation of palaeoproductivity recorded in the calcite of rudist bivalves. The changes in these records are in antiphase with periodic changes in Sr/Ca, indicating that Sr/Ca in V. vesiculosus is lowest in the high-productivity season, which points towards a relationship of Sr/Ca either with temperature or growth rate in this shell. Detrital inclusions in the outer shell layer hamper the full interpretation of trace element records and more specimens need to be studied to examine the expression of these trace element records in rudist calcite.

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Sano, Y.


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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. R Script used to generate FFT power spectra on multi-proxy data according to Muller & MacDonald (2002).

Data S2. Calculations of attenuation length of X-Rays into calcium carbonate for different photon energies, according to: http://henke.lbl.gov/optical_constants/atten2.html