Effect of organic matrices on the determination of the trace element chemistry (Mg, Sr, Mg/Ca, Sr/Ca) of aragonitic bivalve shells (Arctica islandica) — Comparison of ICP-OES and LA-ICP-MS data

BERND R. SCHÖNE,1* ZENGJIE ZHANG,1 DORRIT JACOB,1 DAVID P. GILLIKIN,2 THOMAS TÜTKEN,3 DIETER GARBE-SCHÖNBERG,4 TED MCCONNAUGHEY5 and ANALÍA SOLDATI1

1Institute of Geosciences and Earth System Science Research Center, University of Mainz, Johann-Joachim-Becherweg 21, 55128 Mainz, Germany
2Department of Earth Science and Geography, Vassar College, Poughkeepsie, NY 12604, U.S.A.
3Steinmann Institute of Geology, Mineralogy and Palaeontology, University of Bonn, Poppelsdorf Castle, 53115 Bonn, Germany
4Institute of Geosciences, University of Kiel, Ludewig-Meyn-Straße 10, 24118 Kiel, Germany
52906 Norman Dr., Boise, ID 83704, U.S.A.

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The element chemistry of biogenic carbonates can provide important data on past environments. However, the Sr/Ca and Mg/Ca ratios as well as the Mg and Sr concentrations of biological carbonates, especially aragonitic bivalves often depart from apparent thermodynamic equilibrium. When measured in situ by means of LA-ICP-MS, the Mg concentration is often substantially enriched (two- to threefold) near the organic-rich, annual growth lines. To test the hypothesis that some organic components exert a major influence on the skeletal metal content, the element chemistry of different shell components (insoluble organic matrix, IOM; dissolved CaCO3 and soluble organics, SOM) of Arctica islandica was measured by means of ICP-OES and LA-ICP-MS. The ICP-OES data indicate that the IOM is strongly enriched in Mg (130 ppm) and depleted in Sr and Ca (10 ppm and 0.22 wt%, respectively) when compared to the whole biomineral (Mg: 68 to 99 ppm, Sr: 860 to 1,060 ppm, Ca: ~35.72 wt%). Although the average relative abundance of the IOM barely exceeds 0.46 wt%, its chemical composition in combination with its heterogeneous distribution across the shell can significantly increase estimates of the Mg concentration if measured in situ by LA-ICP-MS. Depending on the distribution of the IOM, the Ca concentration may be significantly lower locally than the average Ca concentration of the whole shell (35.72 wt%). If this remains undetected, the Mg concentration of shell portions with higher than average IOM content is overestimated by LA-ICP-MS and, conversely, the Mg concentration is underestimated in shell portions with lower than average IOM content. Removal of the IOM prior to the chemical analysis by LA-ICP-MS or mathematical correction for the IOM-derived magnesium concentrations is therefore strongly advised. The different chemistry of the IOM may also exert a major control on the trace element to calcium ratios. Shell portions enriched in IOM will show up to 200 times higher Mg/Ca and up to two times higher Sr/Ca ratios than the average shell of A. islandica. Without removal of the IOM prior to the analysis, Mg/Ca and Sr/Ca ratios of shell portions with higher IOM content cannot be used as paleothermometers. Because it is currently impossible to remove the IOM prior to chemical analyses by LA-ICP-MS, we recommend the use of wet chemical techniques (= possibility to separate and measure individual shell components) such as ICP-OES at the expense of lower sampling resolution. The results of this study will significantly improve our understanding of shell-based climate and environmental proxies.

Keywords: trace elements, bivalve shell, organics, LA-ICP-MS, ICP-OES, sclerochronology

INTRODUCTION
Sr/Ca and Mg/Ca ratios of biogenic carbonates can provide quantifiable data on ambient water temperature during biomineralization. This has been empirically dem-

*Corresponding author (e-mail: schoeneb@uni-mainz.de)
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crease (Kinsman and Holland, 1969; Gaetani and Cohen, 2006), while those of abiogenic calcites increase (Katz, 1973; Mucci, 1987). In addition, strontium in calcite is strongly controlled by precipitation rate (Kinsman and Holland, 1969). The orthorhombic crystal structure of aragonite best accommodates the larger Sr2+ ion, while the rhombohedral crystal structure of calcite best accommodates the smaller Mg2+ ion. Therefore, aragonite often contains about 100 times more strontium than does calcite. Nonetheless, Sr/Ca and Mg/Ca ratios in either polymorph of calcium carbonate can provide serviceable paleothermometers.

However, the Sr/Ca and Mg/Ca ratios of biological carbonates often depart from apparent thermodynamic equilibrium. Mg/Ca ratios of some calcitic (Dodd, 1965; Lorens and Bender, 1977; Freitas et al., 2005; Lorrain et al., 2005; Lazareth et al., 2007) and aragonitic bivalve shells (Takesue and van Geen, 2004) are lower than predicted by thermodynamics. Shell Mg/Ca and Sr/Ca ratios can also vary contradictorily among different species and even among conspecific and contemporaneous specimens from one locality (corals: Cardinal et al., 2001; brachiopods: England et al., 2007; bivalves: Dodd, 1965; Gillikin et al., 2005a; Lorrain et al., 2005; Freitas et al., 2008). Dodd (1965) also reported an inverse relation between temperature and Sr/Ca ratios in the nacreous (aragonitic) shell layer of Mytilus edulis, while Gillikin et al. (2005a) observed the opposite in the aragonitic shell of Saxidomus gigantea. The ratios of trace elements to calcium show little consistency in the long-lived bivalve mollusk, Arctica islandica (Toland et al., 2000). Skeletal Mg and Sr contents sometimes correlate with skeletal growth (corals: de Villiers et al., 1995; bivalves: Swan, 1956; Takesue and van Geen, 2004; Gillikin et al., 2005a Lorrain et al., 2005), and also with ontogenetic age (Palacios et al., 1994; Freitas et al., 2005). Such findings suggest that non-thermodynamic factors (e.g., an active, protein-mediated removal of Mg from the inorganic carbonate phase during biomineralization) influence the incorporation of trace elements into biogenic carbonates.

It has also been suggested that organics may influence the skeletal metal content, but such relations have not been quantified (Allison, 1996; Nürnberg et al., 1996; Watanabe et al., 2001; Dauphin et al., 2003; Takesue and van Geen, 2004; Foster et al., 2008). Organics occur both within (intracrystalline; Pokroy et al., 2006; Jacob et al., 2008) and between (intercrystalline) CaCO3 crystals. During shell formation, these organic components mediate biomineralization (Lowenstam, 1981; Veis, 2003). The main biopolymers of bivalve mollusks are water-insoluble structural proteins (β-chitin), water-soluble polyanionic proteins and silk-like proteins (Levi-Kalisman et al., 2001; Sudo et al., 1997). Some of these proteins can be enriched in certain trace elements, foremost magnesium (Cowan, 1991). Nürnberg et al. (1996) estimated that up to 5% of Mg in bulk foraminiferan tests may be associated with proteins rather than with calcite. Likewise, Watanabe et al. (2001) showed that up to 40% of the magnesium in coral aragonite is not lattice-bound. Trace elements released from organic components during sample preparation or measurement may then increase estimates of Mg concentrations and Mg/Ca ratios of biominerals. The metal contents of bivalve organs and tissues have been examined (e.g., Bustamante and Miramaud, 2004), but no study has yet quantified the Mg and Sr content of isolated shell organic matrices and CaCO3 phases.

Analytical techniques such as laser ablation—inductively coupled plasma—mass spectrometry (LA-ICP-MS) and ion microprobe are now widely used to study the skeletal composition in situ. LA-ICP-MS especially offers undeniable advantages with respect to spatial sampling resolution and sample throughput. But combining results for CaCO3 crystals and organic matrices potentially complicates interpretations (Watanabe et al., 2001). Only a few studies have compared results from LA-ICP-MS with wet chemical techniques that separate the organic and inorganic phases (Rosenheim et al., 2005; Gillikin et al., 2005a).

This paper quantifies the organic matrix and CaCO3 fractions in shells of A. islandica and presents the Mg, Sr and Ca concentrations and Mg/Ca and Sr/Ca ratios for both phases, using wet chemical techniques combined with inductively coupled plasma—optical emission spectrometry (ICP-OES). It compares these results with LA-ICP-MS analyses. The benefits of special pretreatment methods and mathematical data corrections are then addressed. The results improve our understanding of trace element chemistry of biogenic skeletons, and can pave the road toward better climate and environmental proxies.

**Material and Methods**

Eight specimens of Arctica islandica were used in the present study (Table 1). The bivalves were collected alive by dredging from ca. 25 to 55 m of water depth northwest of Iceland and from the North Sea (Table 1). Soft tissues were removed, and ligament and periostracum were physically abraded. The outer ca. 1000 μm of each valve were also mechanically removed to eliminate adhering sediment and shell materials possibly altered by early diagenesis. The valves were then ultrasonically rinsed with de-ionized water.

**Sample preparation for chemical analyses of different shell components**

Five specimens were chosen (ICE06-A1 to A5; Table
1) for the analysis of Mg, Sr and Ca contents of the different shell components, i.e., the insoluble organic matrix (IOM), the soluble organics (SOM = sugars, proteins etc.) and the inorganic calcium carbonate component (CaCO₃). The shells were ultrasonically rinsed multiple times in millipore (18.2 MΩ) water, dried and weighed. Both valves of specimens ICE06-A1 and ICE06-A2 and one valve of each of the remaining specimens (whole, uncrushed valves) were gently dissolved in 5% ultrapure HNO₃ over several days before centrifuging the solution at 3700 RPM for 30 min. We used a diluted acid to ensure that water-insoluble organics (IOM) were not dissolved and could be analyzed separately. Then, the IOM of each solution was extracted and rinsed multiple times in millipore water, air-dried and weighed. The IOM of one valve of specimens ICE06-A1 and ICE06-A2 were mounted on glass slides and their chemical composition determined by means of LA-ICP-MS (Table 1). The other IOM samples were completely digested in a 1:1 mixture of 30 vol% ultrapure H₂O₂ plus 65 vol% ultrapure HNO₃ at 90°C (Bellotto and Miekeley, 2007).

After extraction of the IOM, the remaining fluid consisted of SOM and dissolved CaCO₃. For two specimens (Table 1), the SOM with molecular sizes larger than 3kDa (SOM lp) was separated from SOM with proteins smaller than 3kDa (SOM sp) plus dissolved CaCO₃ by ultrafiltration. To achieve this, the fluids were centrifuged at 3,700 RPM for more than 90 min in Vivispin filters (Sartorius, 20 ml). Additionally, the solutions containing SOM lp were also rinsed in millipore water. In order to prevent precipitation of trace elements, we added 1 ml 65% ultrapure HNO₃ to each of the dissolved shell components (IOM, SOM + CaCO₃, SOM lp, SOM sp + CaCO₃). All wet fractions were weighed and then analyzed in a Spectro Ciros Vision ICP-OES at the University of Mainz.

Preparation of shell cross-sections for analyses of crystal fabric and chemistry

One valve of each of the remaining three specimens was mounted on a plexiglass cube and a quick-drying epoxy resin applied to the outer and inner valve surface along the axis of maximum growth (Table 1). On that axis, one or two ca. three-millimeter-thick sections were cut from each valve with a Buehler low-speed saw. The cross-sectioned slabs were mounted on glass slides, ground on glass plates with 800 and 1200 grit powder and finally polished on a Buehler G-cloth with 1 μm Al₂O₃ powder. Prior to the analyses, all samples were ultrasonically rinsed in millipore water.

Chemical analyses of shell cross-sections (LA-ICP-MS and ICP-OES)

One polished cross-section of each of the three specimens HM-Fla86-A1, WH241-597-A1R and DBG13.2-A1

Table 1. List of Arctica islandica specimens used in the present study. IOM = water-insoluble organic matrix, SOM = water-soluble organic matrix, SOM lp = molecular sizes larger than 3kDa, SOM sp = molecular sizes smaller than 3kDa.
Fig. 1. Seasonal strontium and magnesium concentration and Mg/Ca and Sr/Ca ratios in cross-sectioned shell slab of Arctica islandica (specimen WH241-597-A1; Table 1). Data were obtained by LA-ICP-MS. Spots measured 100 μm (long axis) in the direction of growth. Strongly enriched Mg content was observed at reproduction (“R”) lines that formed during warm summer temperatures. These Mg peaks stood out significantly from values measured between consecutive reproduction lines. A slightly higher Mg value also occurred at the “W” line that formed during January/February. The Mg curve had a saw-toothed appearance, whilst the Sr values formed a relatively smooth curve with far less outstanding peaks at “R” and “W” lines. After sampling, the shells were immersed in Mutvei’s solution that stained portions with a higher organic content deep blue (dark grey in black and white print) and those with less organics light blue (light grey). Note de-colored craters (white to light grey) around laser ablation spots. dog = direction of growth. Error bars in standard deviation units (1σ).

Fig. 2. Seasonal strontium and magnesium concentration and Mg/Ca and Sr/Ca ratios in cross-sectioned shell slab of Arctica islandica (specimen DBG1.2-A1; Table 1). See description in caption of Fig. 1. dog = direction of growth. Error bars in standard deviation units (1σ). LA spot size is 50 μm in diameter.
Table 2. Results of chemical analyses of different shell components by means of LA-ICP-MS and ICP-OES. The chemistry and trace element to calcium ratios of the IOM deviated significantly from that of the other shell components such as CaCO$_3$ and SOM (last four columns from left). Results on IOM chemistry revealed by ICP-OES and LA-ICP-MS were statistically invariant (Wilcoxon t-statistics: p > 0.05) if a Ca value of 0.22 wt% (given by ICP-OES) was assumed for the internal standard for LA-ICP-MS. However, if it remained unperceived that the laser coincidentally hit a shell portion with pure IOM, LA-ICP-MS would return unrealistically high Mg and Sr values. For meaning of abbreviations see captions of Table 1. Errors are given as standard deviation (1σ).

<table>
<thead>
<tr>
<th>Rel. abundance (wt%)</th>
<th>Whole biomineral 100</th>
<th>Calculated whole biomineral 100</th>
<th>IOM 0.46</th>
<th>CaCO$_3$ + SOM 99.54</th>
<th>Calculated CaCO$_3$ + SOM 99.54</th>
<th>CaCO$<em>3$ + SOM$</em>{sp}$ 91.95</th>
<th>SOM$_{lp}$ 7.59</th>
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<td>Sr (ppm)</td>
<td>860 ± 128</td>
<td>1,060 ± 170</td>
<td>10 ± 8</td>
<td>1,064 ± 171</td>
<td>820 ± 273</td>
<td>799 ± 435</td>
<td>1,051 ± 370</td>
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<td>Ca (%)</td>
<td>35.72 ± 3.47</td>
<td>35.88 ± 2.82</td>
<td>0.22 ± 0.15</td>
<td>36.02 ± 2.82</td>
<td>36.51 ± 1.98</td>
<td>37.01 ± 2.91</td>
<td>30.18 ± 9.64</td>
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<td>Mg/Ca (mmol/mol)</td>
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<td>0.31 ± 0.09</td>
<td>100.42 ± 67.96</td>
<td>0.31 ± 0.09</td>
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<td>0.35 ± 0.11</td>
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<td>Sr/Ca (mmol/mol)</td>
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<td>1.35 ± 0.22</td>
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<td>1.35 ± 0.22</td>
<td>1.03 ± 0.41</td>
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<th>ICP6-A1+2</th>
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<td>Mg (ppm)</td>
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<td>134 ± 121</td>
<td>21,757 ± 19,669</td>
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<td>Sr (ppm)</td>
<td>864 ± 59</td>
<td>7 ± 1</td>
<td>1,137 ± 133</td>
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<tr>
<td>Ca (%)</td>
<td>35.72</td>
<td>0.22</td>
<td>35.72</td>
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<tr>
<td>Mg/Ca (mmol/mol)</td>
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<td>100.52 ± 90.63</td>
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<tr>
<td>Sr/Ca (mmol/mol)</td>
<td>1.07 ± 0.06</td>
<td>1.50 ± 0.20</td>
<td>1.46 ± 0.17</td>
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</table>
was analyzed by LA-ICP-MS (Table 1). Laser ablation spots in the outer shell layer measured 50 μm in diameter in specimens HM-Fla86-A1 and DBG13.2-A1, and 100 μm in WH241-597-A1R. Specimens DBG13.2-A1 and WH241-597-A1R were used to study the intra-annual (seasonal) variations of trace elements. Therefore, only the youth portions of these shells were sampled, i.e., the shell portions that formed when the bivalves grew at the fastest rates. Each laser spot represented a shell portion that formed within days or a few weeks. In order to analyze the average trace element content, however, a shell portion near the ventral margin of specimen HM-Fla86-A1 was analyzed. The centers of individual laser spots were only approx. 3.45 μm apart from each other and formed a line of measurements (Fig. 4). The lifespan of this bivalve exceeded 200 years, and the average annual increment width at the ventral margin was narrower than the diameter of a laser spot. Thus, each LA-ICP-MS sample from this specimen represented at least one year.

From the other polished slab of specimen HM-Fla86-A1, aragonite powder was milled under a binocular microscope from the same shell portion that was analyzed by LA-ICP-MS (Table 1). We employed a cylindrical diamond drill bit (1 mm diameter, Komet/Gebr. Brasseler GmbH & Co. KG model No. 835 104 010) mounted on a Rexim Minimo drill. Spatial milling resolution parallel to the growth lines was about 55 μm. Each sample swath (55 μm × 1.8 mm) stretched from near the outer shell surface and toward the inside of the shell (approximately 2.8 mm away from the original outer shell surface; it should be noted that the LA-ICP-MS samples were located approx. 1.5 to 2 mm away from the original outer shell surface, i.e., ca. in the middle of the OES-sample swath; Fig. 4). Each of the shell powder samples weighed ca. 150 to 400 μg. Powder samples were completely dissolved in 1 ml 70 vol% ultrapure HNO3 and visually confirmed to be without residue before dilution in 4 ml millipore water. These samples were analyzed in a Spectro CIROSSCP SOP ICP-OES at the University of Kiel.

Biochemical staining and analysis of the shell crystal fabric

In order to identify the distribution of organic components of the shell and study the crystal fabric (Fig. 3), the remaining polished slab of specimen DBG13.1-A2 and the cross-section of WH241-597-A1R that was previously used for LA-ICP-MS were immersed in Mutvei’s solution for 25 and 2 min, respectively, at 37–40°C under constant stirring (Schöne et al., 2005a). Immediately afterward, the etched sections were rinsed in de-ionized water and allowed to air-dry. Mutvei’s solution consists of 0.5 vol% acetic acid, 12.5 vol% glutardialdehyde and ca. 5 g Alcian Blue per liter solution. The acid gently etches the carbonate portions, while the glutardialdehyde preserves the organic matrix in three dimensions. Simultaneously, the Alcian Blue stains organic components deeply blue. Mutvei’s solution is adequate for resolving shell internal growth structures. For scanning electron microscopy (SEM; Hitachi S 4300) the etched shell section of DBG13.1-A2 was sputter-coated with a 30 Å gold layer.

LA-ICP-MS analyses

Solid shell material (Figs. 1, 2 and 4; Table 2) and IOM (Table 2) were analyzed for Mg, by measurement of the isotope 24Mg, and Sr as 88Sr by LA-ICP-MS at the University of Mainz. Ablation was achieved with a NewWave Research UP-213 Nd:YAG laser ablation system (New Wave Research), using a pulse rate of 4–10 Hz, a pulse energy of ~0.3 mJ per spot, and 50 and 100 μm spot diameters with Ar (or a He/Ar mixture) as ablation gas. Analyses were performed on an Agilent 7500ce ICP-MS coupled to the UP-213 platform (one point per peak and 10 ms dwell time) following methods described in Jacob (2006). SRM NIST SRM 612 was used as the external standard (Pearce et al., 1997), and the U.S. Geological Survey glass standard BCR-2G was measured to monitor accuracy and instrumental drift. 43Ca was used as the internal standard with Ca concentrations measured by ICP-OES. Detection limits generally range between 0.001 and 0.5 ppm for these elements. Relative standard deviations (based on repeated measurements of the external standard) were 7.2% for Mg and 7.8% for Sr.

ICP-OES analyses

Dissolved shell samples of five specimens (ICE06-A1 to A5; Table 2) were analyzed with a Spectro CIROS Vision ICP-OES system at the University of Mainz and sample HM-Fla86-A1 with a Spectro CIROSSCP SOP ICP-OES at the University of Kiel (Table 2). We followed the techniques described by Schrag (1999) and de Villiers et al. (2002). Relative standard deviations (triplicate measurements of each sample) were 0.99% for Ca, 1.39% for Mg, and 1.34% for Sr; accuracy for these elements was better than 0.5%. Mixed standard solutions were prepared from single element standards of Mg, Sr and Ca in proportions to reflect those observed in bivalve shells and organic components.

RESULTS

According to our findings, shells of Arctica islandica consist, on average, of 99.54 wt% calcium carbonate (CaCO3) and water-soluble organic matrix (SOM), and 0.46 ± 0.19 wt% water-insoluble organic matrix (IOM) (Table 2). In what follows, CaCO3 + SOM + IOM are referred to as “whole biomineral”.
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Distribution of organics across the shell of *Arctica islandica*

As shown by the sample immersed in Mutvei’s solution for two minutes. Cross-acicular crystal fabric (CA) prevails between major growth lines, whereas irregular simple prismatic crystal fabric (ISP) is observed at the growth line. Note that CA crystal sizes decrease prior to growth line. Because each crystal is surrounded by IOM, decreasing crystal sizes near growth lines result in relative enrichment of IOM and greater resistance of respective shell portions against etching. Therefore, these shell portions stand out as ridges. After the growth line, IOM is depleted, crystals are larger and the shell is more deeply etched. dog = direction of growth.

**Fig. 3.** SEM image of shell portion *Arctica islandica* (specimen DBG1.2-A1) near major annual growth line (reproduction line). Prior to coating with Au, sample was immersed in Mutvei’s solution for two minutes. Cross-acicular crystal fabric (CA) prevails between major growth lines, whereas irregular simple prismatic crystal fabric (ISP) is observed at the growth line. Note that CA crystal sizes decrease prior to growth line. Because each crystal is surrounded by IOM, decreasing crystal sizes near growth lines result in relative enrichment of IOM and greater resistance of respective shell portions against etching. Therefore, these shell portions stand out as ridges. After the growth line, IOM is depleted, crystals are larger and the shell is more deeply etched. dog = direction of growth.

Small-scale (LA-ICP-MS) element variation across the shell of *Arctica islandica*

Magnesium and strontium concentrations in the outer layer of *A. islandica* shells determined by LA-ICP-MS exhibited seasonal oscillations with sharp Mg excursions (Figs. 1 and 2). Highest magnesium values of 106 and 238 ppm (μg g⁻¹) and strontium values of 1387 and 1466 ppm (Figs. 1 and 2) were observed near the major growth lines ("R") of two different specimens. These values corresponded to Mg/Ca ratios of 0.49 and 1.10 mmol/mol
and Sr/Ca ratios of 1.78 and 1.88 mmol/mol. Major growth lines (=reproduction lines; Jones, 1980) form about one month after the hottest part of summer (Fig. 1; Schöne et al., 2005b). A second magnesium peak occurred at growth lines denoted as “W” for winter (Fig. 1). These lines were laid down during ca. January/February (Schöne et al., 2005b). Lowest magnesium (45 and 55 ppm = Mg/Ca of 0.21 and 0.26 mmol/mol) and strontium (845 and 740 ppm = Sr/Ca of 1.08 and 0.95 mmol/mol; Figs. 1 and 2) lev-

els, however, occurred in shell portions that formed during the coldest season of the year, i.e., shortly after the “W” lines. Whereas strontium data formed relatively smooth curves, the magnesium curves demonstrated a saw-toothed appearance with two to three times higher values in samples that comprise major growth lines than samples from shell portions between two consecutive lines (Figs. 1 and 2).

Element analyses of Arctica islandica shell by LA-ICP-MS and ICP-OES

Contemporaneously deposited shell portions analyzed with different analytical techniques revealed statistically indistinguishable results for Mg and Sr as well as Mg/Ca and Sr/Ca ratios (Wilcoxon t-statistics; p > 0.05) (Table 2; Fig. 4). According to ICP-OES analysis, the average Ca concentration in these shell portions was 35.72 wt%. This value was used as the internal standard for LA-ICP-MS.

Fig. 5. Model to estimate the relative abundance of soluble organics in shells of Arctica islandica. The Ca content of the whole biomineral was determined by ICP-OES and equals 35.72 wt% (Table 2). This value limits the pure CaCO₃ content to 89.21 wt%. Accordingly, the minimum amount of soluble organics equals 10.33 wt% (100 wt%−0.46 wt% IOM−89.21 wt% CaCO₃). In this case, the SOM would contain no Ca. With increasing amounts of Ca in the SOM, the relative abundance of the SOM in the biomineral increases and the CaCO₃ content decreases. The hypothetical extreme is where the “biomineral” is CaCO₃-free and consists only of organics, i.e., 0.46 wt% IOM and 99.54 wt% SOM.

Compound-specific chemical analyses by ICP-OES and LA-ICP-MS

Average ICP-OES-derived magnesium concentrations of the IOM (130 ppm; specimens ICE06-A1 to A5) can be up to two times as high as the whole biomineral or the soluble components (68 ppm; Table 2). However, strontium and calcium contents were much lower in the IOM compared to the biomineral (10 ppm and 0.22 wt%, respectively; Table 2). No statistical difference was found between Mg and Sr values or the Mg/Ca and Sr/Ca ratios of the IOM determined by ICP-OES and LA-ICP-MS if a Ca value of 0.22 wt% (= the Ca content in the IOM measured with ICP-OES) was used as the internal standard (Table 2).

Separation of soluble organics with large (SOMlp) and small proteins plus CaCO₃ (CaCO₃ + SOMsp) was not successful (Table 2). As demonstrated by a Ca concentration of 30.18 wt%, the SOMlp component still contained large amounts of dissolved CaCO₃. Overall, water-soluble organics (SOMlp and CaCO₃ + SOMsp) were largely within the same range of magnesium and strontium concentrations as the whole biomineral (Table 2).

Soluble organics: relative abundance and Ca concentration

The relative abundance of SOM in shells of A. islandica can be computed as follows. According to ICP-OES, the whole biomineral contained 35.72 wt% Ca (Table 2). This value represents the sum of the relative abundances of the three main components of the shell, CaCO₃, IOM and SOM, and their respective Ca contents.

\[
35.72 \text{ wt}\% = \frac{a}{100} \cdot A + \frac{b}{100} \cdot B + \frac{c}{100} \cdot C, \quad (1)
\]

where \(a\), \(b\) and \(c\) are the relative abundance in wt% of the IOM, CaCO₃ and SOM, respectively, and \(a = 0.46\) wt%, and \(c = 100\) wt%−\(a−b\). \(A\), \(B\) and \(C\) denote the Ca content (wt%) of the IOM, CaCO₃ and SOM, respectively, and \(A = 0.22\) wt% Ca (given by ICP-OES) and \(B = 40.04\) wt% Ca (stoichiometrical value for abiogenic CaCO₃). We can rewrite Eq. (1) as follows:

\[
35.72 \text{ wt}\% \text{ Ca} = \frac{0.46 \text{ wt}\%}{100} \cdot 0.22 \text{ wt}\% \text{ Ca} + \frac{b}{100} \cdot 40.04 \text{ wt}\% \text{ Ca} + \frac{100 \text{ wt}\% - 0.46 \text{ wt}\% - b}{100} \cdot C, \quad (2)
\]

and solve Eq. (2) for \(C\).
by Watanabe

non-lattice-bound magnesium in coral skeletons reported organic components or crystal surfaces. The amount of that 40% or the total skeletal magnesium is absorbed by and adhering metals of coral skeletons. They concluded treatment procedures to remove the organic components also are complexated by proteins (Mitterer, 1978).

Portions of coral skeletons and suggested that these met-
allothioneins or phosphoproteins (Margoshes and Vallee, 1957; Noel-Lambot, 1976; Marsh and Sass, 1985). A common detoxification system is based upon soluble complexes etc.) with magnesium-binding capacities (Gómez-Ariza et al., 2004; Gotliv et al., 2005). Actually, Mg is one of the most abundant cofactors of metalloproteins (Dudev and Lim, 2000) and occurs at negatively charged sites of aspartic and glutamic-rich polypeptides (Dudev et al., 1999). These two amino acids belong to the most common constituents of glycoproteins of the molluscan extrapallial fluid (EPF) and play an active role in promoting and modulating shell mineral growth (Sikes et al., 1998; Gotliv et al., 2003). Later during biomineralization, these acidic macromolecules mainly become part of the intracrystalline matrix (Addadi et al., 1991) while other framework building insoluble components are mainly preserved as intercrystalline matrix. Furthermore, similar proteins sequestrate and remove metal ions from metabolic pathways. This is necessary because excessive amounts of such ions (mainly derived from food, Chapman et al., 2003) can have adverse effects on organisms. Different metal cation detoxification systems have therefore been developed in organisms (Viarengo and Nott, 1993). A common detoxification system is based upon soluble ligands, i.e., metal-binding proteins such as metallothioneins or phosphoproteins (Margoshes and Valle, 1957; Noel-Lambot, 1976; Marsh and Sass, 1985). These proteins may become part of the EPF.

Some proportions of Mg may merely co-occur with the IOM. During biomineralization, Mg is largely excluded from aragonite crystals and is therefore enriched in the peripheral fluids surrounding the crystals. In this case, Mg does not necessarily bind to the IOM, but may be adsorbed with the IOM (or occur as a Mg enriched Mg–Ca–CO₃ surface coating on the carbonate crystals). However, the use of a low-concentration acid (HNO₃) in the present study to dissolve the biominerals lower Mg/Ca ratios in subfossil Mercenaria spp. analyzed with LA-ICP-MS. It is noteworthy, that none of the existing studies differentiated between insoluble and soluble organics.

Most recently, Foster et al. (2008) analyzed shells of Arctica islandica by means of Synchrotron X-ray Absorption Near Edge Spectroscopy (XANES) and concluded that Mg is not substituted into the aragonite of A. islandica, but is exclusively hosted by a disordered phase such as organic components or nanoparticles. According to these authors, Mg does not appear to be a useful paleoenvironmental proxy at all. However, Mg contents in shells of A. islandica approach those of current synchrotron methods. Most Mg levels of shells analyzed in the present study remained between 68 and 99 ppm. Therefore, the conclusions drawn by Foster et al. (2008) seem debatable and require further study.

The affinity of Mg for the IOM may result from the existence of organic molecules (metalloproteins, metal complexes etc.) with magnesium-binding capacities (Gómez-Ariza et al., 2004; Gotliv et al., 2005). Actually, Mg is one of the most abundant cofactors of metalloproteins (Dudev and Lim, 2000) and occurs at negatively charged sites of aspartic and glutamic-rich polypeptides (Dudev et al., 1999). These two amino acids belong to the most common constituents of glycoproteins of the molluscan extrapallial fluid (EPF) and play an active role in promoting and modulating shell mineral growth (Sikes et al., 1998; Gotliv et al., 2003). Later during biomineralization, these acidic macromolecules mainly become part of the intracrystalline matrix (Addadi et al., 1991) while other framework building insoluble components are mainly preserved as intercrystalline matrix. Furthermore, similar proteins sequestrate and remove metal ions from metabolic pathways. This is necessary because excessive amounts of such ions (mainly derived from food, Chapman et al., 2003) can have adverse effects on organisms. Different metal cation detoxification systems have therefore been developed in organisms (Viarengo and Nott, 1993). A common detoxification system is based upon soluble ligands, i.e., metal-binding proteins such as metallothioneins or phosphoproteins (Margoshes and Valle, 1957; Noel-Lambot, 1976; Marsh and Sass, 1985). These proteins may become part of the EPF.
assured that adhesively bound Mg was removed from the IOM prior to the analyses.

**IOM and magnesium distribution across shells of Arctica islandica**

As demonstrated by geochemical staining experiments (immersion in Mutvei’s solution, Fig. 1) and SEM analyses (Fig. 3), organic components, especially the IOM, are not homogeneously distributed across the shells, but are strongly enriched near major growth lines. Mutvei’s solution stained these lines dark blue indicating the presence of large amounts of organic molecules. In addition, SEM analyses revealed that significantly smaller crystals occur near such growth lines compared to other portions of the outer shell layer. Because each crystal is embedded in an organic sheet of IOM, shell portions with smaller crystals contain relatively larger amounts of intercrystalline organics. During biomineralization, these organic matrices provide the structural framework (Clark, 1980) and control the material properties of the biominerals (Mann, 1983; Simkiss and Wilbur, 1989; Crenshaw, 1990; Watabe et al., 1993). Between major growth lines, however, a cross-acicular crystal fabric with larger crystal sizes prevailed, and the amount of insoluble organics in the respective shell portions was much smaller. Immersion of such shell portions in Mutvei’s solution resulted in a mottled fabric consisting of large, light blue (probably intracrystalline organics) stained crystals and scanty intercrystalline organics (darker blue).

**Pitfalls of element analyses of Arctica islandica shells by LA-ICP-MS**

If the precise Ca concentration of the analyzed material is known, LA-ICP-MS returns trace element values that are statistically indistinguishable from those measured by ICP-OES. For example, using the observed Ca concentration of 0.22 wt% (Table 2) as an internal standard, LA-ICP-MS and ICP-OES returned similar Mg and Sr data for the IOM (Table 2). This finding also suggests that potential interferences by organics on mass 24 have only minor effects on the Mg measurements. If organic molecules would influence the data, LA-ICP-MS values for the Mg concentration would be significantly different from those measured by ICP-OES. Likewise, a direct comparison of similar shell portions analyzed by LA-ICP-MS and ICP-OES revealed statistically indistinguishable trace element concentrations (Table 2). It should be noted that the analyzed samples came from an ontogenetically old shell portion. Low calcification rates in such shell portions resulted in narrow annual increments. Each sample taken from such locations represented more than a year of shell growth precluding the analysis of seasonal variations of trace elements. Given the relatively low sampling resolution, the distribution of the IOM (and the Ca level; here: 35.72 wt%) in such shell portions can be considered to be nearly homogeneous.

However, fast-growing youth portions of bivalve shells can be analyzed with sub-annual resolution by LA-ICP-MS. In such shell portions, the Mg concentration (and Mg/Ca ratios) near major growth lines (=larger amounts of IOM) were two- to threefold higher than in neighboring shell portions with lower IOM content (Figs. 1 and 2). A likely explanation for the overestimation of the Mg concentration of shell portions enriched in IOM is a significant Mg contribution from the larger amounts of IOM present. In most existing LA-ICP-MS studies of biogenic hard parts, the small-scale heterogeneous distribution of the IOM across the shell and its chemical composition has received little attention. Instead of determining the precise Ca concentration of each shell portion designated for element analyses by means of LA-ICP-MS, it is often standard practice to measure the Ca concentration of the entire shell and use this value as the internal standard. However, the IOM to CaCO₃ ratio across the shell is highly variable, particularly in youth portions of the shell, and shell portions containing more IOM evidently contain less CaCO₃ and, thus, have a lower Ca content. It should be noted that it is currently very difficult to determine the Ca concentration of the sample volume ablated for LA-ICP-MS, because the sample volume excited by the electron microprobe for Ca measurements is much smaller. In spots, in which the amount of IOM exceeds 0.46 wt% of the total sample weight, this can result in a significant overestimation of the Mg and Sr concentration, whereas lower than average amounts can result in an underestimation of these elements. This will be demonstrated by a hypothetical model (Fig. 6).

In this model, we use a Ca content of 35.72 wt% as internal standard value (Ca concentration given by multiple ICP-OES measurements; Table 2) for all calculations. In the two extreme cases, (a) ablation of an IOM-free shell portion and (b) coincidental ablation of a purely organic spot, the Mg and Sr concentrations will be underestimated (a) or overestimated (b), respectively. In case (a), an unchanged internal standard value that does not take into account the admixture of virtually Ca-free, but Mg- and Sr-rich IOM would result in an overestimation of the actual Mg and Sr concentrations (Mg = 134 ppm; Sr = 7 ppm) by 16,139% and 16,400%, respectively (Mg = 21,761 ppm; Sr = 1,155 ppm; Table 2). A mixing model for these two end members, 0 wt% and 100 wt% IOM, provides a means to estimate the potential error induced by different amounts of IOM in the biomineral (Fig. 6). For example, if a shell portion containing 50 wt% insoluble organics is ablated, apparent Mg and Sr values would be as high as 237 and 866 ppm, respectively. These hypothetical considerations demonstrate that a local enrichment of IOM can lead to a significant overestimation of
the Mg level of the sampled shell portion if the actual Ca concentration is not precisely determined. Precisely how the IOM concentration varies across the shell on μm- and nm-scales remains to be studied.

**Trace element to calcium ratios of *Arctica islandica* shells**

So far, our analyses have demonstrated that element concentrations of homogeneous shell portions and isolated IOM measured by ICP-OES and LA-ICP-MS agree well. Furthermore, the Mg/Ca and Sr/Ca ratios calculated from the respective ICP-OES and LA-ICP-MS data were statistically identical (Table 2). However, as shown in Figs. 1 and 2, high-resolution LA-ICP-MS analysis of fast-growing youth portions of the shells not only resulted in higher Mg values near major, IOM-enriched growth lines, but also in elevated Mg/Ca ratios. This is likely to be due to the different chemical composition of the IOM and the CaCO₃ + SOM components. In the model in Fig. 7, the effect of increasing amounts of IOM on the Mg/Ca and Sr/Ca ratios is simulated. If the analyzed shell portions consist of pure IOM, Mg/Ca and Sr/Ca ratios are ca. 100.44 and 1.46 mmol/mol, respectively. However, in an assumed extreme case that the analyzed shell portions consist of a 1:1 mixture (by weight) of IOM and CaCO₃ + SOM, the Mg/Ca and Sr/Ca ratios will amount to 1.10 and 1.11 mmol/mol, respectively. For comparison, the average shell (0.46 wt% IOM) returned Mg/Ca ratios of 0.46 mmol/mol and Sr/Ca ratios of 1.10 mmol/mol. This model suggests that shell portions enriched in IOM can have higher Mg/Ca and—if the IOM content is large enough—higher Sr/Ca ratios. Trace element to calcium ratios of shell portions enriched in IOM may not be useful as paleothermometers, because the relationship between temperature and Mg/Ca and Sr/Ca ratios has only been verified for CaCO₃, but not for the IOM.

**Soluble organics (SOM)**

It is currently very difficult to ascertain the trace elemental composition of the soluble organics or the precise relative abundance of SOMlp and SOMsp in the whole biomineral. Our approach to separate the soluble organics by molecular size was not successful, because the SOMlp fraction apparently still contained large amounts of dissolved CaCO₃ (Table 2). Preliminary data, however, suggest that the chemical composition of the SOM does not vary as much as the IOM from the inorganic shell fraction. Other available techniques to separate the SOM from the CaCO₃ involve cationic ion exchange resin (Albeck et al., 1996). However, these methods involve non-ultrapure agents and are thus inappropriate to determine the trace element composition of the SOM.

It is noteworthy that the relative abundance of SOM in the shells of *A. islandica* was unexpectedly high in
comparison to previous reports. Kawaguchi and Watabe (1993) found only 2.6 wt% IOM + SOM in Crassostrea virginica shells. However, these data came from the prismatic shell layer which may contain considerably less organics than the crystal fabrics of A. islandica. Typically, the prismatic shell layer is only very weakly stained by Mutvei’s solution indicating low amounts of organics (Schöne et al., 2005a). Further studies are required to confirm the amounts of SOM in A. islandica shells.

Implications for geochemical analyses of bivalve shells

As demonstrated here, an indiscriminate use of analytical techniques may produce imprecise data on trace element concentrations of bivalve shells. Strontium levels of bivalve shells can be determined with sufficient precision and accuracy by both ICP-OES and LA-ICP-MS techniques. However, Mg concentrations are severely overestimated by LA-ICP-MS, because (1) the IOM is enriched in Mg and (2) the internal standard value assumed for the whole biomineral may not be appropriate to each shell portion; some shell portions such as near major growth lines can be significantly enriched in IOM and, thus, depleted in Ca compared to the whole biomineral. Without proper sample pretreatment or mathematical modeling, high-resolution LA-ICP-MS-derived Mg concentrations cannot be compared with ICP-OES data. Potential sample pretreatment procedures can include removal of the IOM by oxidative cleaning (see Takesue and van Geen, 2004). However, some of the intracrystalline organic matrix may be insoluble as well and thus difficult to remove (e.g., by roasting; Gaffey et al., 1991), without dissolving the shell (compare Watanabe et al., 2001). Alternatively, the surplus of Mg contained in the IOM can be mathematically corrected for. If the average IOM content and its artifactual Mg concentration were known, its contribution to the Mg concentrations of the whole biomineral could be calculated and subtracted.

The use of trace element to calcium ratios as faithful paleothermometers, however, does not require absolute element concentrations. Even if the exact Ca-concentration of the ablated material is not known due to the heterogeneous nature of the material, Mg and Sr values will change proportionally, and Mg/Ca or Sr/Ca ratios will not be fractionated. However, existing studies (inorganic precipitation experiments) have only confirmed a temperature effect on the Mg/Ca and Sr/Ca ratios of the CaCO₃, while such effects on the IOM have never been studied. Given the different chemical composition of the IOM, shell portions enriched in IOM will not only show higher Mg, Sr and Ca concentrations, but also higher Mg/Ca and Sr/Ca ratios than the CaCO₃ + SOM fraction. This is applicable to both the ICP-OES and LA-ICP-MS techniques. However, wet analytical techniques permit the separation of the different shell components prior to the analysis, whereas a reliable removal of the IOM from the biomineral prior to LA-ICP-MS analysis is not currently established. During LA-ICP-MS analysis the entire shell material is ablated, i.e., IOM, SOM and CaCO₃. Therefore, shell portions with higher relative IOM abundance will return elevated Mg/Ca and Sr/Ca ratios. Without removal of the IOM prior to the analysis, Mg/Ca and Sr/Ca ratios of shell portions with higher IOM content cannot be used as paleothermometers.

SUMMARY AND CONCLUSIONS

The insoluble organic matrix of Arctica islandica shells is significantly enriched in magnesium and depleted in strontium and calcium in comparison with the inorganic carbonate fraction and soluble organics. Although the average relative abundance of the IOM barely exceeds 0.5% by weight, its chemical composition can significantly increase estimates of the Mg content of the shell if measured by LA-ICP-MS. This overestimation is related to the heterogeneous distribution (on µm- and nm-scales) of the IOM across the shells. It is currently still very difficult to determine the Ca concentration (used as internal standard) of the exact same volume that is ablated for LA-ICP-MS. Thus, Mg concentrations of shell portions with higher than average IOM content, such as major growth lines, are prone to be overestimated by LA-ICP-MS. Removal of the IOM prior to the chemical analysis or mathematical correction for the IOM-derived magnesium concentrations is strongly advised.

For paleoenvironmental reconstructions, however, it is necessary to determine the element to calcium ratios of the CaCO₃ component. Existing studies (inorganic precipitation experiments) have only demonstrated a temperature effect on the Mg/Ca and Sr/Ca ratios of the CaCO₃, but not of the IOM. Without removal of the IOM prior to the analysis, Mg/Ca and Sr/Ca ratios of shell portions enriched in IOM cannot be used as paleothermometers. Because it is currently not possible to remove the IOM prior to LA-ICP-MS analysis, we recommend the use of wet chemical techniques such as ICP-OES at the expense of lower sampling resolution.

The trace metal chemistry of soluble organics in biminerals still requires further study. Our preliminary approach only focused on molecular sizes of the SOM above and below 3kDa and separation of these two phases from dissolved CaCO₃ was incomplete. Chromatographic separation methods (Mazon et al., 1990) may help to determine the Mg content in different molecule size classes as well as the relative abundance of each different SOM class in the whole biomineral. Such data may help to define mathematical models that can eliminate the potential overestimation of Mg by means of LA-ICP-MS.
Similar influences of organic proteins on chemical estimates are also expected for other metals that are often bound to organic molecules such as Co, Fe, Mn, Mo, Ni, Se and Zn (e.g., Lochmüller et al., 1974; Gómez-Ariza et al., 2004). Further studies should therefore isolate different organic components of the shell and determine their amount and chemical composition by wet chemical analyses.

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