Disentangling the biological and environmental control of *M. edulis* shell chemistry

Agnes Heinemann, Claas Hiebenthal, Jan Fietzke, Anton Eisenhauer, and Martin Wahl

Leibniz-Institut für Meereswissenschaften (IFM-GEOMAR), Wischhofstraße 1-3, D-24148 Kiel, Germany (aheinemann@ifm-geomar.de)

[1] Blue mussel individuals (*Mytilus edulis*) were cultured at four different salinities (17, 20, 29, and 34). During the course of the experiment, temperature was gradually increased from 6°C to 14°C. Mg/Ca and Sr/Ca ratios of the shell calcite portions produced during the 9 weeks of experimental treatment as well parts that were precipitated before the treatment phase were measured by laser ablation–multicollector–inductively coupled plasma–mass spectrometry. Mg/Ca ratios show a positive correlation with temperature for individuals cultured at salinity 29 and 34 (Mg/Ca (mmol/mol) ∼ (0.2–0.3)*T (°C)), while for individuals cultured at low salinities (17, 20) no trend was observed. Sr/Ca ratios were not affected by temperature but strongly by salinity. The data show very strong biological influence (“individual differences” and “physiological variability”) on elemental ratios (79% on Mg/Ca and 41% on Sr/Ca) in *M. edulis* calcite. The results challenge the use of blue mussel shell data as environmental proxies.

Components: 3900 words, 6 figures, 2 tables.

Keywords: *Mytilus edulis*; proxy archive; shell chemistry; biological control.

Index Terms: 0424 Biogeosciences: Biosignatures and proxies; 1065 Geochemistry: Major and trace element geochemistry; 1050 Geochemistry: Marine geochemistry (4835, 4845, 4850).

Received 23 August 2010; Revised 5 January 2011; Accepted 4 February 2011; Published 29 March 2011.


1. Introduction

[2] Understanding climate history helps interpreting fluctuations or directional shifts of parameters like temperature or CO₂ in the context of global change. Biological climate archives are valuable records of environmental conditions during the lifetime of the organism. Mussels have the potential to provide high resolution records of a large variety of environmental regimes due to their high growth rate, global distribution [Gosling, 1992] and tolerance to a broad range of environmental conditions [Seed and Suchanek, 1992] and some bivalves are extremely long-lived [e.g., Schöne et al., 2005]. Trace metal distributions (e.g., Mg and Sr) in bivalve shells are a field of current interest because as a proxy archive [e.g., Klein et al., 1996a, 1996b; Lazareth et al., 2003; Immenhauser et al., 2005; Freitas et al., 2008; Wanner et al., 2008], they may help reconstructing (paleo)environmental conditions and thus deliver the data necessary to construct and improve models of past climate.

[3] However, several recent studies [e.g., Klein et al., 1996b; Vander Putten et al., 2000; Gillikin et al. 2005; Carré et al., 2006; Freitas et al. 2006; Heinemann et al., 2008] on elemental ratios
in bivalve shells failed to show unambiguous relationships between elemental ratios and environmental factors (e.g., temperature and salinity). The reasons are often referred to as “biological control” or “vital effects” meaning elemental ratios in the mussel shell (e.g., Mg/Ca, Sr/Ca) are not exclusively controlled by inorganic thermodynamic principles but also by individual physiological processes. For example, Klein et al. [1996b] suggested that skeletal chemistry of Mytilus trossulus from the field is primarily controlled by rate of mantle metabolic activity (metabolic pumping of Ca to the extrapallial fluid (EPF)) and only secondarily modified by variation of seawater salinity. Also, in their study on the distribution of Mg, Sr and Pb in the calcite shell layer of Mytilus edulis, Vander Putten et al. [2000] showed that patterns of these elements cannot be explained by seasonal variations in seawater composition alone. According to the latter study the direct use of Mg as a proxy in M. edulis shells is hampered by the absence of a consistent Mg-temperature relationship over the year. Crystal growth rate in two aragonitic marine bivalve species (Mesodesma donacium and Chione subrugosa) was found to be the main factor influencing trace elements concentrations, especially for Sr (explaining up to 74% of the variance) [Carré et al., 2006]. As a consequence, the environmental control on minor and trace elements in mollusks is often too weak to develop suitable proxies [Carré et al., 2006]. The findings from recent bivalve studies corroborate the results of previous studies reporting a strong biological influence and the interaction among different environmental factors affecting elemental ratios in bivalve shells [e.g., Heinemann et al., 2008].

The ambiguity of results in recent studies raises the question as to whether the influence of a single environmental factor on bivalve shell composition can be strong enough to provide a reliable signal superimposing the “noise” produced by other environmental variables and/or by biological effects. Hence, it is important to quantify the influence of single environmental variables such as temperature or salinity as well as the combined effect of such factors on elemental ratios (i.e., Sr/Ca and Mg/Ca) relative to the biological background “noise” such as genetics, physiology, growth and reproductive cycles and infections, respectively. In the present study we investigated the influence of temperature, salinity, individual differences and physiological variability on element to calcium ratios in the calcite layer of juvenile blue mussels (M. edulis) to critically assess its suitability as a proxy archive.

2. Materials and Methods

2.1. Culturing

Young (6–9 mm) blue mussels, Mytilus edulis, collected in August 2003 from settlement panels deployed in the Kiel Fjord in spring of the same year, were first kept under identical and controlled conditions in a flow through system of filtered seawater (salinity around 17) in the laboratory at 12°C for 5 months. During this time, the bivalves were fed daily with natural plankton caught from Kiel Fjord (20–100 μm) at concentrations typical for the Baltic Sea (3 × 10^3 cells/mL) [Clausen and Riisgard, 1996].

After this laboratory acclimatization phase, the juvenile M. edulis were partitioned into 4 treatment groups in 2 L aquaria. Subsequently, the bivalves were gradually adapted (salinity change by 5 units per week), to 4 different salinities (17, 20, 29, 34). Salinities higher than 17 (initial salinity) were obtained by adding artificial marine salt (Tropic Marin, Dr. Biener, Wartenberg) to natural Baltic Seawater. In this experimental setup, each experimental unit consisted of the 2 L aquarium (replicate) containing 20 mussel individuals of similar size (pseudo replicates) and 2 reservoirs. A pump placed in a lower reservoir filled an upper reservoir, allowing the water to flow through the culture tanks (2 L aquaria) back into the lower reservoir. The setup resulted in a total water volume of ~75 L for each experimental unit. The water was exchanged once per week.

As in the Baltic Sea blue mussels’ growth rates are normally highest in early spring and summer [Kautsky, 1982a], the experiment was conducted from 4 February to 13 May 2004. In the course of the experiment temperature was continuously adjusted to the seasonal Kiel Fjord water temperature (20 year average of the experimental period February until May) [Lehmann et al., 2002] and ranged from 6°C to 14°C. In this treatment phase, food consisted of cultured algae (Dunaliella sp. and Rhodomonas sp.) at a concentration of 6 × 10^5 cells/mL. Prior to the daily feeding, the algal suspension was adjusted to experimental salinity and constantly dripped (20 mL/min) into the aquaria over a period of 2 h (72 × 10^5 cells/mussel). Light exposure was 10 h a day (~150 μE/m^2/sec). For more details on culturing technique used see the
study of Kossak [2006]. Growth for each individual mussel was monitored by measuring shell length (from umbo to shell margin, longest distance) at five dates (4 February, 9 and 25 March, 22 April, and 13 May). At the end of the experiment, the size range was 11–14 mm.

### Table 1. Instrument Parameters of the LA-ICP-MS Measurements

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Explanation of Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AXIOM MC-ICP-MS</strong></td>
<td></td>
</tr>
<tr>
<td>Cool gas</td>
<td>14 L/min</td>
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<tr>
<td>Auxiliary gas</td>
<td>1.5 L/min</td>
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<tr>
<td>Nebulizer gas</td>
<td>0.6 L/min</td>
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<tr>
<td>RF power</td>
<td>1000 W</td>
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<tr>
<td>Reflected power</td>
<td>2 W</td>
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<tr>
<td>Ion energy</td>
<td>4968 V</td>
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<tr>
<td>Cones</td>
<td>R.A. Chilton RAC19/RAC705</td>
</tr>
<tr>
<td>Resolution</td>
<td>500 res</td>
</tr>
<tr>
<td>Integration time per scan</td>
<td>2 s</td>
</tr>
<tr>
<td>Measured isotopes (Faraday Cup used)</td>
<td>$^{24}\text{Mg}(L3)$, $^{25}\text{Mg}(Ax)$, $^{26}\text{Mg}(H3)$, $^{44}\text{Ca}(L4)$, $^{46}\text{Ca}(Ax)$, $^{48}\text{Ca}(H4)$, $^{84}\text{Sr}(L2)$, $^{85}\text{Rb}(L1)$, $^{86}\text{Sr}(Ax)$, $^{87}\text{Sr}(H1)$, $^{88}\text{Sr}(H2)$</td>
</tr>
<tr>
<td><strong>UP193 Solid State Laser</strong></td>
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<tr>
<td>Ablation gas</td>
<td>0.6 L/min (He)</td>
</tr>
<tr>
<td>Spot size/distance between spots</td>
<td>35 μm/100 μm</td>
</tr>
<tr>
<td>Fluence</td>
<td>3 J/cm$^2$</td>
</tr>
<tr>
<td>Repetition rate</td>
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</tr>
<tr>
<td>Ablation time</td>
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<tr>
<td>Washout time</td>
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</table>

### Table 2. Means and Variances of Shell Parts Grown in Nature Under Similar Conditions Before the Actual Treatment Started$^a$

<table>
<thead>
<tr>
<th></th>
<th>Salinity 17</th>
<th>Salinity 20</th>
<th>Salinity 29</th>
<th>Salinity 34</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mg/Ca (mmol/mol)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean</td>
<td>6.2</td>
<td>8.0</td>
<td>7.7</td>
<td>7.8</td>
</tr>
<tr>
<td>variance</td>
<td>1.6</td>
<td>3.0</td>
<td>2.5</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>Sr/Ca (mmol/mol)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean</td>
<td>1.379</td>
<td>1.55</td>
<td>1.54</td>
<td>1.47</td>
</tr>
<tr>
<td>variance</td>
<td>0.001</td>
<td>0.01</td>
<td>0.02</td>
<td>0.01</td>
</tr>
</tbody>
</table>

$^a$Grouped with respect to the treatment applied.

### 2.2. Sample Preparation

[8] At the end of the treatment phase, mussels were sacrificed by freezing (−18°C) and their soft body removed. From each salinity treatment the shell of one individual (randomly selected among the survivors of the 20 pseudo replicates per experimental unit) was embedded in a two component epoxy resin (Buehler, EPO-THIN, Low Viscosity Epoxy Resin). When the resin had hardened, shells were repeatedly cut along the axis of maximum length using a microtome saw (Leica SP 1600) producing 200 μm thick sections.

Figure 1. Growth of mussels during the experiment. Length was measured at five dates. Distance between laser spots was 100 μm and their location was calculated by the known points of growth. Line shows temperatures during experimental phase [Kossak, 2006].
Ca, Sr and Mg profiles were measured in the \( x_m = 2 \) tests. To reduce the probability of \( M. trossulus \)\([\text{Klein et al., 2008}]\) individuals cul-
where \( n \) is the number of

\( \Delta \) is the average elemental

\( X \) is the average elemental ratio of all 4 individuals) and (2) the mean variance of elemental ratios of the 4 single individuals over different points in time during the phase of constant abiotic conditions,

\[ s_{\text{phys}} = \frac{1}{n} \left( \sum_{i=1}^{n} (x_i - \bar{x})^2 / (n - 1) \right) / n, \]

the 6°C phase, “physiological variability,” where \( n \) is the number of measurements within one individual \( i \) and \( x_i \) is the elemental ratio of the measure \( i \).

The influence of abiotic factors was calculated (1) by subtracting the value of the individual differences from the mean variance among the individuals cultured at different salinities during 6°C phase (“salinity”),

\[ s_{\text{sal}} = s_{\text{ind}}(6\text{C-phase}) - s_{\text{ind}}. \]

and (2) by subtracting the physiological variability from the average variance over the time while temperature increased (\( t_{\uparrow} \)-phase, “temperature”),

\[ s^2_{\text{t}} = s^2_{\text{phys}(t_{\uparrow})} - s^2_{\text{phys}}. \]

To reveal possible significant differences between contributions of different factors (physiological variability, individual differences, temperature, salinity) to the overall variance, we performed multiple \( F \) tests. To reduce the probability of type 1 errors, the significance level (alpha) was Bonferroni-corrected.

3. Results and Discussion

\[ \text{Klein et al., 1996a} \] reported that skeletal Mg/ Ca ratios of \( M. trossulus \) grown in the field provide an accurate measure of temperature and that weekly sea surface temperature may be estimated with an apparent accuracy of approximately \( \pm 1.5 \)°C. In the shells of \( M. edulis \) individuals cultured at high salinities (29, 34), we also observed Mg/Ca ratios in the calcite layer to be positively related to temperature (Figures 3a and 4). However,
this pattern was not found in shells of individuals cultured at lower salinities (17, 20). Wanamaker et al. [2008] found a more robust Mg/Ca temperature relationship at salinity 23 and 28 than at higher salinity (32) in their study on Mg/Ca and Sr/Ca ratios in the calcite of juvenile *Mytilus edulis*. They concluded that Mg/Ca and Sr/Ca ratios in juvenile *M. edulis* calcite may possibly be used for reconstruction of water temperatures from an upper estuarine setting. For mean values of the investigated salinity range (23–32), Wanamaker et al. [2008] observed an increase of 6%/°C for Mg/Ca and 1%/°C for Sr/Ca, respectively. The results of Mg/Ca are nearly similar to the results of the overlapping salinity range of this study (29, 34) where a temperature relation of 6.5%/°C was
found. The different findings for Sr/Ca may be a result of adaptation and/or physiology as our animals originated from a low salinity habitat (∼18) the ones cultured by Wanamaker et al. [2008] came from a salinity of ∼31. Furthermore, we investigated a smaller temperature range of 6°C–14°C in contrast to 7°C–19.3°C. Our results partly correspond with previous findings where Mg/Ca ratios of different taxa are also reported to be influenced by salinity. Dodd [1965] showed that the Mg concentration in the outer prismatic layer of M. edulis increases markedly with decreasing salinity and that it shows a weak positive correlation with temperature. Further, studies on foraminifera demonstrate that variations in temperature and salinity are reflected in the magnesium content of foraminifera calcite [e.g., Nürnberg et al., 1996]. Their experiments reveal that pronounced salinity changes (≥10) at constant temperature significantly affect the Mg concentrations. Nürnberg et al. [1996] therefore suggested that Mg may serve as a paleoproxy for past surface water temperatures, as long as salinity variations and post depositional alterations like carbonate dissolution can be shown to be small or absent. Our results likewise show that temperature interacts with other factors influencing Mg/Ca ratios in the shell of M. edulis. Strikingly, individual differences among mussels (34.0%) living under similar environmental conditions and temporal variability within individuals in constant environmental conditions (45.2%) contribute a substantially higher proportion to the overall variance of the Mg/Ca ratio than temperature does (20.8%) (Figure 6a). As the influence of the physiological state of the bivalves on Mg/Ca incorporation into bivalve shells is likely to vary with environmental fluctuations as well as growth and reproductive cycles, food availability, fouling or infection rates, our findings may explain the absence of a consistent Mg/Ca-temperature relationship over the year in M. edulis calcite observed by Vander Putten et al. [2000]. Additionally, as variances of Mg/Ca ratios among individuals decreased after placing the mussels into different salinity treatments relative to variability of Mg/Ca in shell parts grown in nature.
indicates that a putative salinity effect on Mg/Ca is smaller than interindividual differences in a fluctuating natural environment. Consequently, no influence of salinity on the Mg/Ca ratio which was strong enough to emerge from the biological background “noise” could be detected. While Mg/Ca tended to be highest in the two low salinity treatments individuals’ shells (SAL 17 and 20, Figure 4), partly corroborating Dodd’s [1965] results a reliable Mg/Ca-salinity effect was not apparent.

[12] Sr/Ca ratios in M. edulis shells were not significantly affected by temperature (Figures 3b, 5, and 6b). The temperature contribution to the overall variance (0.3%) was significantly smaller than the contribution of physiological state (24.4%), individual differences (16.9%) and salinity (58.5%) (F test, p < 0.0001). Therefore, we cannot corroborate findings of Dodd [1965] who found that the strontium concentration in M. edulis calcite correlates positively with temperature. Instead, we found the salinity treatment to have the highest impact on Sr/Ca (58.5%, Figure 6b). We observed the highest Sr/Ca value in the shell grown at the lowest salinity (SAL 17). However, no significant Sr/Ca-salinity relationship was detected (Figure 5).

4. Conclusion

[13] We observed relations between Mg/Ca ratios and temperature in two of the measured Mytilus edulis shells. Still, biological control (physiological state and individual differences) has a dominant influence (~79% contribution to overall variance on Mg/Ca and ~41% contribution on Sr/Ca) on the elemental ratios in M. edulis shells. This can be taken as a likely reason for the large discrepancies in the discussions whether carbonates of bivalve shells can be used as proxy archives. It appears that mussel carbonate can only be used as a proxy archive if the combination of environmental conditions and the major contribution of biology are considered. Therefore, the data stress the importance of replicating at the biological level, i.e., measure several animals from the same location and time, even though this drastically increases the measuring effort.

Acknowledgments

[14] We gratefully acknowledge Ute Kossak for providing mussel shells. The paper also benefited from the constructive reviews of Joel Baker and an anonymous reviewer. Finally, we thank Marghaleray Amini and Florian Böhm for their interest in this study and for fruitful discussions.

References


Clausen, I., and H. Riisgard (1996), Growth, filtration and respiration in the mussel Mytilus edulis: No evidence for physiological regulation of the filter-pump to nutritional

Figure 6. Influence of individual differences, physiological state, salinity, and temperature on (a) Mg/Ca and (b) Sr/Ca ratios in the prismatic layer of M. edulis shells. Salinity contribution on Mg/Ca was not detectable.


