Temperature dependence of aragonite and calcite skeleton formation by a scleractinian coral in low mMg/Ca seawater

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ABSTRACT
Temperature-dependent aragonite and calcite formation by scleractinian corals were examined in low molar (m) Mg/Ca seawater, the experimental conditions replicating the fluctuating mMg/Ca levels prevailing throughout the Phanerozoic Eon. Incubation and skeletal growth monitoring of juveniles of the scleractinian coral Acropora solitaria were for 4 months from the planula stage, in seawater with mMg/Ca ratios of 5.2, 1.0, and 0.5, and temperatures of 19–28 °C, indicated that polymorphism of present-day scleractinian corals in low mMg/Ca seawater is also influenced by seawater temperature. However, corals produced more aragonite than formed in inorganic CaCO₃ precipitation experiments under the same conditions, except at 19 °C. Although the aragonite content reflected the results of the latter (abiotic) experiments at 19 °C, it is suggested that aragonitic scleractinian corals controlled skeletal formation biologically under low mMg/Ca conditions at higher temperature, growth rates being faster at 25 °C and slower at 19 °C for all mMg/Ca ratios. Compared with growth rates with the present-day-equivalent seawater Mg/Ca level of 5.2, juvenile growth decreased by 62.8% ± 14.7% and 56.7% ± 6.7% under mMg/Ca levels of 1.0 and 0.5, respectively; the results suggest that growth of aragonitic scleractinian corals is suppressed throughout varying seasonal temperatures under low mMg/Ca conditions. This supports previous findings from variable temperature perspectives that scleractinian corals grow more slowly in low mMg/Ca (Cretaceous) seawater, interpreted as a possible explanation for the hiatus in scleractinian reef building in the Cretaceous Period.

INTRODUCTION
The molar Mg/Ca ratio (mMg/Ca) of seawater has varied throughout the Phanerozoic Eon (541 Ma to the present day), including three episodes of aragonite/high-Mg calcite-facilitating conditions (mMg/Ca > 2) and two of calcite-facilitating conditions (mMg/Ca < 2), known as aragonite and calcite seas, respectively (Sandberg, 1983; Hardie, 1996; Ries, 2010). It has been proposed that the skeletal mineralogy of newly evolved calcifying organisms, such as the selection of aragonite versus calcite, was largely dictated to by the mMg/Ca in seawater at the time when mineralized skeletons were first acquired (Porter, 2007, 2010). The fossil record shows that the reign of scleractinian corals was interrupted during the mid-Cretaceous Period when mMg/Ca dropped to its lowest levels, creating conditions that were unfavorable for corals with aragonitic skeletons (Stanley, 2003), a Cretaceous scleractinian coral (Coelosmilia sp.) having been discovered with a calcitic skeleton (Stolarski et al., 2007). Nevertheless, aragonitic coral skeletons from this period have also been reported (Sorauf, 1999). In addition, Janiszewska et al. (2017) described a Cretaceous phase in the fossil coral record in which scleractinian micrabaciid corals appeared with aragonitic skeletons, and Kiessling et al. (2008) reported that large-scale patterns of coral skeletal mineralogy have been affected by mass extinction events. Although confirmable amounts of calcite have been reported in present-day coral skeletons grown in seawater of low Mg/Ca (mMg/Ca < 1.5), the major skeletal composition is aragonite, with maximum calcite levels of 20% in mMg/Ca of 0.5 (Higuchi et al., 2014) and as much as 36% calcite in mMg/Ca of 1.5 and 1.0 (Ries et al., 2006).

In the Cretaceous Period, mMg/Ca levels dropped and temperatures increased simultaneously, seawater temperatures in the mid-Cretaceous being higher than in present-day oceans (Wilson and Norris, 2001; Forster et al., 2007). Experimental studies on bimineralic mussels showed that a 2 °C temperature increase with constant mMg/Ca results in a decrease in aragonite secretion, with little change in that of calcite (Fitzer et al., 2014). De Choudens-Sanchez and Gonzalez (2009) showed that polymorph mineralogy is controlled by a combination of the saturation state of CaCO₃ and the mMg/Ca ratio in solution, abiogenic precipitation experiments having suggested that polymorphism of CaCO₃ is controlled by mMg/Ca as well as temperature (Morse et al., 1997). Balthasar and Cusack (2015) showed experimentally that in the case of inorganic precipitation, aragonite content increased at a higher temperature with high mMg/Ca, whereas calcite content increased at a lower temperature with low mMg/Ca. However, the biological responses of skeletal organisms to environmental changes during the Phanerozoic Eon are still unknown; there have been no experimental studies on the effects of synergistic changes in both temperature and mMg/Ca using skeletal organisms. As suggested by Kiessling (2015), studies on the skeletal formation of CaCO₃ during manipulation of both temperature and mMg/Ca are required. Here we report the temperature dependence of aragonite and calcite formation by scleractinian corals in low mMg/Ca seawater.

METHODS
Incubation of Juvenile Corals
Planula larvae of Acropora solitaria were collected at the Biological Institute of Kuroshio, Kochi, Japan, and incubated in seawater with manipulated mMg/Ca ratios under controlled temperatures. The larvae were maintained in each experimental condition for 3 days and then induced to metamorphose using the neuropeptide 2 µM Hym 248 (Iwao et al., 2002), subsequently settling in plastic vessels as juveniles. After the introduction of symbiotic algae, Symbiodinium spp. (CCMP 2556, Clade D), to the juveniles (Yuyama and Higuchi, 2014), manipulated seawater was changed every 2–3 days, and juveniles were observed for 4 months under each experimental condition. Four electrically thermoregulated incubators (THS030PA; Advantec) with LED lighting (100 µmol m⁻² s⁻¹, light:dark = 12 h:12 h) were set to temperatures of 19, 22, 25, and 28 °C for the experiment, the average temperatures inside the incubators, recorded using a temperature logger, being 19.3 ± 0.5 °C, 21.7 ± 0.4 °C, 25.1 ± 0.5 °C, and 27.6 ± 0.6 °C.

Juvenile corals were incubated in manipulated seawater with three different concentrations of Mg and Ca (Mg/Ca = 2.5, 1.2, 0.5), prepared by mixing filtered (pore size 0.22 µm) natural seawater and Mg-free water, the
latter having been prepared by dissolving NaCl, Na2SO4, CaCl2, KCl, KBr, SrCl2, NaF, H3BO3, and NaHCO3 in ultrapure water. The concentrations of major elements were manipulated to mimic those of natural seawater, excluding Mg (i.e., no addition of Mg). During incubation for 4 months, the mg/MgCa ratios were 5.15 ± 0.02, 0.94 ± 0.05, and 0.46 ± 0.01, as determined by inductively coupled plasma–mass spectrometry (ICP-MS). The measured values were compared to International Association for the Physical Sciences of the Oceans standard seawater (Ocean Scientific International Ltd, UK), the precision for mg/MgCa measurement being <0.7%. The pH of the manipulated seawater was 8.0–8.1, total alkalinity was 2.2–2.3 mmol kg⁻¹, and salinity was 32.0–32.3 psu. After incubation, the juvenile corals were treated with NaClO to remove tissue and the skeletons were used for estimation of growth rate and determination of their crystal structure. Growth rates were estimated on a dry weight basis for 5–8 juveniles under each treatment condition at the end of the incubation, the initial skeletal weight being zero.

**RESULTS**

XRD analysis showed that both calcite and aragonite were formed by scleractinian Acropora corals under the manipulated seawater conditions (Table 1). In mg/MgCa = 5.2 (equivalent to present-day natural seawater), pure aragonite skeletons were formed under all temperatures examined. The aragonite content became lower (i.e., calcite content higher) in lower mg/MgCa treatment groups, although still remaining high (even at mg/MgCa = 1.0), except at the lowest temperature (19 °C; Table 1). However, higher calcite content resulted under lower temperatures. Aragonite peaks following XRD analysis were obscured due to the strong calcite peaks with mg/MgCa = 1.0 at 19 °C, in addition to mg/MgCa = 0.5 at 22 °C and 25 °C (Fig. DR2); the presence of aragonite was confirmed with Meigen's stain (Fig. 1). These analyses indicated that calcite content was >90%, the corresponding aragonite content being <10% (Table 1). At mg/MgCa = 0.5, calcite formed the major skeletal component, although ~45% aragonite was coproduced at 28 °C. The skeletons were composed entirely of calcite at mg/MgCa = 0.5 (19 °C); aragonite was not evidenced by either XRD or Meigen’s stain (Fig. 1; Fig. DR2). However, the settlement success rate of planula larvae with decreased mg/MgCa at 19 °C was very low, especially with mg/MgCa = 0.5 (<1% success). Under the other experimental conditions, A. solitaryensis showed relatively high success rates for settlement and growth, even under conditions of low mg/MgCa or low temperature (e.g., mg/MgCa < 1 at 22 °C; mg/MgCa = 5.2 at 19 °C). However, juvenile coral growth rates decreased significantly at low mg/MgCa under each temperature condition examined (p < 0.05; Table 2), being fastest at 25 °C and slowest at 19 °C for all Mg/Ca ratios. Compared with those in mg/MgCa = 5.2, the average juvenile growth rates decreased by 62.8% ± 14.7% and 56.7% ± 6.7% at mg/MgCa levels of 1.0 and 0.5, respectively, under all temperature conditions.

**DISCUSSION**

Growth rates were fastest at 25 °C for all mg/MgCa conditions examined. Although the precipitation rate of CaCO3 (both aragonite and calcite) is theoretically faster at higher temperatures (Burton and Walter, 1987), in the case of corals biological processes also affect calcification. A temperature of 25 °C is considered optimal for growth of A. solitaryensis, which is distributed in temperate waters off Japan. The present growth

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**TABLE 1. ARAGONITE AND CALCITE CONTENT IN BIOGENIC (CORAL ACROPORA) AND ABIOTIC CALCIUM CARBONATE IN DIFFERENT TEMPERATURE AND MOLAR Mg/Ca REGIMES**

<table>
<thead>
<tr>
<th>Table 1.</th>
<th>Temperature (°C)</th>
<th>19</th>
<th>22</th>
<th>25</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acropora solitaryensis (this study)</td>
<td>mg/MgCa = 5.2</td>
<td>A + A</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>mg/MgCa = 1.0</td>
<td>A + C</td>
<td>A + C</td>
<td>A + C</td>
<td>A + C</td>
</tr>
<tr>
<td></td>
<td>mg/MgCa = 0.5</td>
<td>C</td>
<td>A + C</td>
<td>A + C</td>
<td>A + C</td>
</tr>
</tbody>
</table>

**Note:** mg/MgCa is MOLAR Mg/Ca. A is 100% aragonite, C is 100% calcite, A + C is mixture of aragonite and calcite. Numbers in brackets indicate aragonite content (%). Mean ± standard error (n = 3).

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**Figure 1. Juveniles of Acropora solitaryensis (Meigen’s stain).** Purple stained regions are aragonite; uncolored regions are calcite. A: 28 °C, mg/MgCa = 5.2. B: 28 °C, mg/MgCa = 0.5. C: 25 °C, mg/MgCa = 0.5. D: 22 °C, mg/MgCa = 0.5. E: 19 °C, mg/MgCa = 0.5. Scale bars are 0.5 mm.
rate data for lower temperatures correspond to those of previous studies that indicated that the calcification rate of *A. solitaryensis* was lower at 18 °C than 23 °C (Higuchi et al., 2015).

As in a previous report on inorganic precipitation (Balthasar and Cusack, 2015), polymorphism of CaCO₃ by corals in low mMg/Ca seawater is shown here to be dependent upon seawater temperature. Kiessling’s (2015) linear regression model to estimate temperature \((T, \text{in} °C)\) from known percentages of abiogenic aragonite \((A)\) and seawater mMg/Ca \((R^2 = 0.73)\), when converted to determine aragonite percentages \([i.e., A = (T + 8.5 \times \text{mMg/Ca} – 26.6 / 0.18)]\), shows that most of the present results were above the line of theoretical inorganic precipitation of aragonite (Fig. 2). Thus, corals produced more aragonite than expected under inorganic conditions, for each experimental regime except at 19 °C. Polymorphism of CaCO₃ in biomineralizing organisms is controlled by organic matrix proteins (Belcher, 1996; De Yoreo and Dove, 2004; Goffredo et al., 2011), and the higher than expected aragonite proportions in corals for most experimental scenarios are most likely a reflection of this biological influence.

However, our experiments also demonstrate that at very low mMg/Ca (0.5) coral skeletal mineralogy is dominated by calcite or, at 19 °C, even when converted to determine aragonite percentages \([i.e., A = (T + 8.5 \times \text{mMg/Ca} – 26.6 / 0.18)]\), most of the results were above the line of theoretical inorganic precipitation of aragonite (Fig. 2). Thus, corals produced more aragonite than expected under inorganic conditions, for each experimental regime except at 19 °C. Polymorphism of CaCO₃ in biomineralizing organisms is controlled by organic matrix proteins (Belcher, 1996; De Yoreo and Dove, 2004; Goffredo et al., 2011), and the higher than expected aragonite proportions in corals for most experimental scenarios are most likely a reflection of this biological influence.

Figure 2. Aragonite content in *Acropora* cultured under different temperature and mMg/Ca regimes. Circle symbols—mMg/Ca = 5.2; open triangles—mMg/Ca = 1.0; open squares—mMg/Ca = 0.5; mean ± standard error \((n = 3)\) (all *A. solitaryensis*; this study); solid triangle—mMg/Ca = 1.0; solid square—mMg/Ca = 0.5 (both *A. tenuis*; Higuchi et al., 2014). Dashed line indicates mMg/Ca = 1.0; solid line indicates mMg/Ca = 0.5 (by linear regression model \(A = (T + 8.5 \times \text{mMg/Ca} – 26.6 / 0.18)\) \((T\) is temperature); modified from Kiessling, 2015). Plots above lines indicate higher proportion of aragonite skeleton than inorganic precipitation of aragonite.

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