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An Experimental Approach to Assessing the Roles of Magnesium, Calcium, and Carbonate Ratios in Marine Carbonates

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Abstract: Marine biomineralization is a globally important biological and geochemical process. Understanding the mechanisms controlling the precipitation of calcium carbonate [CaCO$_3$] within the calcifying fluid of marine organisms, such as corals, crustose coralline algae, and foraminifera, presents one of the most elusive, yet relevant areas of biomineralization research, due to the often-impenetrable ability to measure the process in situ. The precipitation of CaCO$_3$ is assumed to be largely controlled by the saturation state [Ω] of the extracellular calcifying fluid. In this study, we mimicked the typical pH and Ω known for the calcifying fluid in corals, while varying the magnesium, calcium, and carbonate concentrations in six chemo-static growth experiments, thereby mimicking various dissolved inorganic carbon concentration mechanisms and ionic movement into the extracellular calcifying fluid. Reduced mineralization and varied CaCO$_3$ morphologies highlight the inhibiting effect of magnesium regardless of pH and Ω and suggests the importance of strong magnesium removal or calcium concentration mechanisms. In respect to ocean acidification studies, this could allow an explanation for why specific marine calcifiers respond differently to lower saturation states.

Keywords: marine biomineralization; inorganic mineralization; coral reefs; ocean acidification (OA); omega; dissolved inorganic carbon (DIC); extracellular calcifying fluid (ECF)

1. Introduction

Calcium carbonate (CaCO$_3$) is the most important biogenic mineral, in terms of quantity, global distribution, and diversity [1]. The production of CaCO$_3$ provides a number of ecological goods and services, such as shoreline protection and habitat structures. For example, coral reefs are one of the most important living bioconstructions of CaCO$_3$ [2] harboring one-quarter to one-third of all marine species [3], and thus serving to be socially and economically important [4]. Unfortunately, future projections show marine biomineralization will become severely impacted by ocean acidification (OA) due to the reduction of carbonate ion concentrations in the oceans [5,6].

Corals calcify extracellularly in a fluid that is separated from the seawater by at least two cell layers [7,8] and rely on a number of active and passive ionic exchanges. For example, calcium ions are actively transported into the extracellular calcifying fluid (ECF) by the epithelium cells of the coral polyp [9,10] while protons are removed [11], establishing favorable conditions for the precipitation of CaCO$_3$ [12]. Similarly, carbon either diffuses into the ECF as carbon dioxide [CO$_2$] or is actively transported into the ECF in the form of bicarbonate [13,14]. Some coral species can calcify in ocean water that is undersaturated with respect to aragonite [15], whereas other species cease to grow and vanish [16,17], which demonstrates a range of biological controls governing the mineralization process. Therefore, to understand which marine calcifiers will be affected by future reduction in ocean saturation states and to estimate its implications for the global carbon cycle, we need to explore a range of possible ECF scenarios.
The significance of biologically-induced and biologically-influenced mineralization is irrefutable. For example, the skeletal organic matrix [SOM] within corals is considered a major factor controlling the precipitation of CaCO$_3$. A number of studies have reported that the SOM contains not only acid-rich proteins (e.g., sulphated proteoglycans), but also assemblages of adhesion and structural proteins, which together are thought to provide a template for aragonite precipitation [10,18–20]. Additionally, the dissolution and precipitation of CaCO$_3$ in aqueous solution is largely dependent on abiotic factors relating to the saturation state ($\Omega$) of the ECF [21,22], which is defined by the product of the dissolved ions forming the mineral divided by the stoichiometric solubility product, $K_{sp}^*$ (Equation (1)).

$$\Omega = \frac{[Ca^{2+}]\cdot[CO_3^{2-}]}{K_{sp}^*} \quad (1)$$

As expressed above, $\Omega$ is an extremely useful indicator of the equilibrium or disequilibrium of a solution with a mineral surface. When the ion product, $[Ca^{2+}]\cdot[CO_3^{2-}]$, equals the solubility product, $K_{sp}^*$, the saturation state equals one and the system is in equilibrium. If the saturation state is below one because the ion product is lower than the solubility product, the solution is undersaturated and the mineral dissolves. A saturation state greater than one indicates supersaturation where the product of the ion concentrations is greater than the solubility product. In this case, it is thermodynamically viable that dissolved ions precipitate into a crystal structure [22]. The observation that the precipitation rate of CaCO$_3$ increases with an increasing saturation state [21,23–27] has led to the development of empirical relationships (Equation (2)) that describe the calcification rate, G, as a function of the saturation state, where $k$ is the reaction rate constant and $n$ is the empirical reaction order.

$$G = k[\Omega - 1]^n \quad (2)$$

This equation has been applied to predict calcification rates in corals [28] and has successfully been used to simulate the dynamics of ion concentrations in the calcifying fluid of corals and coccolithophores [14,28–30]. However, Equation (2) appears to ignore the theoretical basis shown in Figure 1, which emphasizes how the product of varying calcium and carbonate ion concentrations can obtain the same $\Omega$ value. This is also supported by [31], which demonstrated how the rate of calcite precipitation differed due to the ratio of calcium to carbonate despite having the same oversaturated $\Omega$. Although $\Omega$ is a good predictor of dissolution and precipitation of CaCO$_3$, it does exclude the possibility that ion concentrations differ while obtaining the same $\Omega$ and could therefore account for observational variations among marine calcifiers.

**Figure 1.** Varying concentrations of calcium and carbonate ions [Ca$^{2+}$:CO$_3^{2-}$] at fixed $\Omega_{ara}$ of 25, 15, and 5. This demonstrates the underlining principle that calcium and carbonate ion concentrations can obtain the same $\Omega_{ara}$ value at different Ca:CO$_3$ ratios, therefore questioning the empirical equation that prescribes the calcification rate as a function of $\Omega_{ara}$ alone.
Based on this rational, we decided to incubate coral skeleton fragments under six controlled abiotic chemo-static scenarios. By emulating previously measured ECF conditions, we kept all the solutions oversaturated in respect to $\Omega_{\text{ara}} = 10$, pH 8.7, and maintained a typical tropical temperature of 25 °C. Experiment 1a recreated a magnesium [Mg] free condition (strong Mg removal activity) with a high Ca:CO$_3$ ratio (e.g., no dissolved inorganic carbon (DIC) concentrating mechanism and a weak proton removal from the ECF), while experiment 1b recreated a Mg-free solution with a low Ca:CO$_3$ ratio (e.g., mimicking a DIC concentrating mechanism resulting in DIC concentrations three times greater than ambient seawater and a strong proton removal from the ECF resulting in elevated total alkalinity ($T_A$) four times greater than ambient seawater). Experiment 2a recreated a medium Mg scenario (representing concentrations half that of the modern seawater) with a high Ca:CO$_3$ ratio, while experiment 2b recreated a medium Mg scenario with a low Ca:CO$_3$ ratio. Experiment 3a recreated a high Mg scenario (equal to that of modern seawater, i.e., no active removal of ions from the ECF) with a high Ca:CO$_3$ ratio, while experiment 3b recreated a high Mg scenario with a low Ca:CO$_3$ ratio.

2. Materials and Methods

2.1. Preparation of the Seed Material

There are a range of methodological approaches used to study CaCO$_3$ precipitation, previous studies have used powdered calcite 3–7 µm diameter as the seeding material [32], or Iceland spar [31], living specimens, e.g., [33,34], or synthetic crystals [35]. We used bioclastic fragments of *Stylophora pistillata* to add a potentially realistic coral aragonite crystal structure and investigate if active ion transport, as mediated by the coral calcifying tissue, suffices to drive coral calcification. The ion transporters of the tissue are simulated via the pumped fluids. Our experiment, therefore, aimed to mimic natural processes. However, the only biological component that was not included in the experiments were the organic molecules. This approach is also comparable to a recently published study that did include organic molecules in the incubations [36]. The seeding material for all experiments was obtained from aquarium grown *Stylophora pistillata* (Leibniz Center for Tropical Marine Research [ZMT], Bremen) and followed the methods of [36]. The coral skeleton fragments were cleaned for 48 h with hydrogen peroxide (H$_2$O$_2$ 30%) to remove any soluble components and organic tissue. The coral skeleton fragments were then rinsed in Millipore® water, dried at 40 °C for 24 h, afterwards ground in a planetary ball mill (PM100, Retsch®) for 1 min, and dry sieved (1–200 µm). Individual bioclastic fragments were then hand-picked under a light microscope and selected based on uniform size and shape. These bioclasts are considered rough and represent a typical biogenic skeleton structure. The heterogenetic nature of coral skeletal structure adds a potentially realistic portrayal of the crystal surface adjustment to the ECF but also adds natural variability that occurs in all treatments. Afterwards, each bioclastic fragment was placed in an individual Eppendorf® Safe-Lock 0.5-mL microcentrifuge tube filled with ethanol and placed in an ultrasonic bath for 5 min to remove residual powder and again dried at 40 °C for 24 h. A by-product of this cleaning procedure could result in an increase of the micro-porosity of the bioclastic fragments, by the removal of organic material or breakage. Each fragment was weighed before and after the incubations on a Mettler Toledo® scale with a 1-µg precision (room humidity 30% and temperature 22 °C). As the size and weight of each bioclastic fragment was not perfectly uniform (0.364–1.449 g; Table A1), all bioclastic fragments were evenly distributed among treatments. The initial and end weights, and standardized daily weight increases can be found in the Appendix A (Table A1). To understand the difference in precipitation rate, a two-way factorial analysis of variance (ANOVA), least square (LS), and Tukey–Kramer honest significance difference (HSD) test of the standardized mean weight change between the six experimental scenarios were performed using the software JMP version 9.0. Microstructure formed during each experimental scenario was identified using a scanning electron microscope (Tescan Vega 3 XMU SEM, ZMT) back-scatter electron (BSE) images. Crystal structures of individual CaCO$_3$ polymorphs (vaterite, calcite and
aragonite) were analyzed under the Raman microscope at the Alfred Wegener Institute for Polar and Marine Research (AWI) in Bremerhaven, Germany, with the help of Dr. Gernot Nehrer. Due to the uneven surface of the incubated crystals, we did not perform a mapping of the whole crystal but focused on individual crystal structures to qualitatively identify the polymorphs with the Raman spectrum (Figure A1).

2.2. Experimental Setup

Ten custom-built incubation chambers made of Teflon [31,36] were used to conduct three cross-factor experiments with two Ca:CO$_3$ scenarios (high and low) in parallel with three Mg concentrations (0 mM, 26.5 mM, and 53 mM) in series (Figure 2; Table 1). Each chamber was attached with Tygon and Marprene$^\text{®}$ tubing to two 1-L Tedlar$^\text{®}$ gas sampling bags filled with either a calcium chloride [CaCl$_2$] or sodium bicarbonate [NaHCO$_3$], the preparation of the stock solution is detailed below. Five replicates were run for each treatment. The volume of each incubation chambers was 0.25 mL. The initial flow rate of the solutions into the chambers was accelerated to quickly fill the incubation chambers and then reduced to a constant flow (10 µL min$^{-1}$) via a 24-channel peristaltic pump (Ismatec$^\text{®}$). The seed material was placed in each of the incubation chambers and the experiments were run between 32–70 days in a temperature-controlled Rumed$^\text{®}$ climate cabinet maintained at a constant 25 °C (±0.5 °C). The variation in experimental duration was due to unexpected health and safety issues of the authors not being allowed into the laboratory.

![Figure 2](image-url)

*Figure 2.* Schematic design of the experimental conditions showing (a) the separate stock solutions of Ca and CO$_3^-$ passing through the peristaltic pump and mixing in the reaction chambers, and (b) the physicochemical conversions of CaCO$_3$, bicarbonate [HCO$_3^-$] and CO$_3$ occurring during mineral precipitation and dissolution phases. Each of the six experimental scenarios is outlined in Table 1.

2.3. Preparation of Stock Solutions

All experiment stock solutions were prepared with Millipore$^\text{®}$ water, which was initially boiled to drive out dissolved CO$_2$ and then kept in a constant N$_2$ atmosphere to prevent CO$_2$ in-gassing. For all stock solutions pH was measured with a WTW-Multi 3430 Set K pH senor and calibrated with the pH 4 and 10 buffers at 25 °C. Among all the experiments temperature, salinity, and pH remained constant at 25 °C, 36, and 8.7, respectively. The aragonite saturation state, $\Omega$ara, in all incubations was 10, with a satura-
tion index, $SI_{ara} = \log(\Omega_{ara})$, of 2.8, which should induce aragonite precipitation. These parameters represent conservative estimates of realistic scenarios for the coral ECF [11,37]. The aquatic properties chosen for the stock solutions in these experiments reflect the ECF parameters known for *Galaxea fascicularis*, but may not be representative for other coral species, e.g., [38,39]. The full details of the quantity of chemical compounds used for each experiment can be found in Table 2. Each chemical compound was weighed on a Mettler Toledo® scale with a 1 µg precision (room humidity 30% and temperature 22 °C). Concentrations of CaCl$_2$ and magnesium chloride [MgCl$_2$] in the calcium stock solution and NaHCO$_3$ in the carbonate stock solution were double the target concentrations for calcium and carbonate ions because the fluids enter the incubation chambers at a 1:1 ratio and dilute each other’s concentration by half (quantiles are given in Table 2A). After adding all the necessary chemical compounds to the stock solutions, they were transferred into 1-L Tedlar® gas sampling bags [36] and put into the climate cabinet at constant 25 °C ($\pm$0.5 °C). This study would improve greatly if microsensors were installed in the incubation chambers to monitor the real time chemistry. Unfortunately, our approach relies on the calculated parameters inside the chambers similar to the work of [31,36].

The calcium stock solutions were prepared by dissolving CaCl$_2$ in 5-L of carbon-free Millipore® water. For the experiments containing magnesium, MgCl$_2$ was added to the stock solution of CaCl$_2$. The Mg concentrations were chosen to represent a strong ion removal mechanism (0 mM, control Mg treatment), a medium ion removal mechanism [26.5 mM, equivalent to half the concentration in present day seawater], and a weak ion removal mechanism (53 mM, equivalent to the concentration in present day seawater). The amount of sodium chloride [NaCl] was then adjusted to maintain a final salinity of 36. Neither carbon nor alkalinity was present in the CaCl$_2$ stock solution (pH = 7), therefore maintaining a zero DIC and $\Delta$A concentration.

The carbonate stock solutions were prepared by dissolving NaHCO$_3$ in 5-L of carbon-free Millipore® water. Sodium hydroxide [NaOH] was added via titration to adjust $\Delta$A and to reach a pH of 8.716. The pH of the carbonate stock solution was 8.716 because when it mixes with the CaCl$_2$ solution (pH = 7) in the incubation chamber the pH will adjust to 8.700 because DIC and $\Delta$A are known to mix conservatively [40]. DIC and $\Delta$A were calculated for equilibrium carbonate chemistry in NaCl using the dissociation constants of [41].

Table 1. Solutions setup for incubation experiments. Calcium, magnesium, and DIC concentrations are arranged by the amounts of CaCl$_2$, MgCl$_2$, and NaHCO$_3$ added to the solutions. Temperature and salinity remained constant at 25 °C and 36 g kg$^{-1}$, respectively. Carbonate concentrations and $\Delta$A are calculated for equilibrium carbonate chemistry in NaCl using the dissociation constants of [41].

| Exp. | Ca$^{2+}$.CO$_3^{2-}$ | Mg$^{2+}$.Ca$^{2+}$ | Ca$^{2+}$ | CO$_3^{2-}$ | Mg$^{2+}$ | $\Omega_{ara}$ | pH | DIC | $\Delta$A |
|------|---------------------|---------------------|---------|-----------|---------|----------------|-----|-----|---------|
|      | mol:mol             | mol:mol             | mM      | µM        | mM      | µM             | µM  | µM  | µM      |
| 1a   | 47                  | 0                   | 10.6    | 226       | 0       | 10             | 8.7 | 1777 | 2440    |
| 1b   | 2.8                 | 0                   | 2.6     | 926       | 0       | 10             | 8.7 | 7270 | 9885    |
| 2a   | 47                  | 2.5                 | 10.6    | 226       | 26.5    | 10             | 8.7 | 1777 | 2440    |
| 2b   | 2.8                 | 10.2                | 2.6     | 926       | 26.5    | 10             | 8.7 | 7270 | 9885    |
| 3a   | 47                  | 5                   | 10.6    | 226       | 53      | 10             | 8.7 | 1777 | 2440    |
| 3b   | 2.8                 | 20.4                | 2.6     | 926       | 53      | 10             | 8.7 | 7270 | 9885    |

Although the concentration of calcium in the ECF is known to vary, previous studies have recorded values between 9–15 mM from cold-water corals 9–12.3 mM with a mean of 9.9 mM; [42] and the tropical corals *Pocillopora damicornis* and *Acropora youngei* range between ca. 9–15 mM; [43]. The target value of 10.6 mM calcium, was chosen for the incubations with a high Ca:CO$_3$ (47:1) stoichiometry because it represents conditions measured with microelectrodes in the ECF of *Galaxea fascicularis* 9–11 mM; [11]. Although the target value of 2.6 mM calcium is perhaps unrealistically low, it was chosen for the incubations with a low Ca:CO$_3$ (2.8:1) stoichiometry to emulate a strong proton removal from the ECF (resulting in elevated $\Delta$A four times greater than ambient seawater), as well
as a DIC concentrating mechanism (three times greater DIC than ambient seawater) as proposed by a number of authors [28,44–46]. These Ca:CO$_3$ stoichiometries were also chosen to maintain constant pH and Ω$_{ara}$ between the treatments.

3. Results

3.1. Precipitation Rates

A highly significant interactive effect of Mg ion concentration and Ca:CO$_3$ concentration was observed (Figure 3; Table 2; $F_{2,24} = 150.924$, $p < 0.001$). When Mg was included into the aquatic solution, neither a significant weight change of the CaCO$_3$ seed nor a difference between the two Ca:CO$_3$ scenarios were observed (Table 2; Table A1). Conversely, both Mg-free Ca:CO$_3$ scenarios had significant weight increases. The Mg-free 47:1 Ca:CO$_3$ scenario had a calcification rate four times that of the Mg-free 2.8:1 Ca:CO$_3$ scenario (Figure 3, points labelled A and B). The average weight increase (± SD) in the Mg$^-$ free 47:1 Ca:CO$_3$ treatment was 1.017 (± 0.130) mg d$^{-1}$ and 0.229 (± 0.061) mg d$^{-1}$ in the Mg-free 2.8:1 Ca:CO$_3$ treatment. The high amount of newly formed CaCO$_3$ measured in the Mg-free 47:1 Ca:CO$_3$$^{2-}$ is partly explained by spontaneous nucleation, which was only observed in this scenario. Slight dissolution was observed under the intermediate (26.5 mM) and high (53 mM) Mg scenario with a 47:1 Ca:CO$_3$ concentration (−0.001 mg d$^{-1}$ ± 0.001 and −0.002 mg d$^{-1}$ ± 0.003, respectively). The intermediate and high Mg scenario with a 2.8:1 Ca:CO$_3$ concentration had no significant weight changes (0.000 mg d$^{-1}$ ± 0.001 and 0.002 mg d$^{-1}$ ± 0.007, respectively), the Ca:CO$_3$ concentration had no effect on the growth rate when Mg ion concentration was equal to or half that found in the ambient ocean, indicating that in this situation Mg has a stronger inhibiting effect towards calcification than Ca:CO$_3$ concentrations.

![Figure 3](image_url)

**Figure 3.** Scatter plot showing the standardized total weight change per day [mg d$^{-1}$] of each experimental scenario. The data shows a clear inhibiting effect of Mg and 2.8:1 Ca:CO$_3$ stoichiometry towards inorganic mineralization. Treatments not connected by the same alphabetical symbol (A, B, or C) are significantly different as shown in Table 2; $F_{2,24} = 150.924$, $p < 0.001$. 

| Source | DF | SS  | MS  | F-Ratio |
|--------|----|-----|-----|---------|
| Model  | 5  | 4.142 | 0.828 | 240.390 <0.001 |
| Error  | 24 | 0.083 | 0.003 |         |

$\text{Mg}^{2+}$ $\cdot$ Ca$^{2+}$:CO$_3^{2-}$ $\pm$ 0.003, respectively). The intermediate and high Mg scenario with a 2.8:1 Ca:CO$_3$ concentration had no significant weight changes (0.000 mg d$^{-1}$ ± 0.001 and 0.002 mg d$^{-1}$ ± 0.007, respectively), the Ca:CO$_3$ concentration had no effect on the growth rate when Mg ion concentration was equal to or half that found in the ambient ocean, indicating that in this situation Mg has a stronger inhibiting effect towards calcification than Ca:CO$_3$ concentrations.
Table 2. Two-way factorial ANOVA and least square (LS) means difference. The effect test showed significant interaction between Ca:CO$_3^-$ and Mg concentration. Tukey–Kramer HSD comparisons indicates a significant difference between Mg-free and high Ca:CO$_3^-$, and all other treatments, as well as Mg-free and low Ca:CO$_3^-$ and all other treatments.

| Source                  | DF | SS   | MS   | F-Ratio | p > F  |
|-------------------------|----|------|------|---------|--------|
| Model                   | 5  | 4.142| 0.828| 240.390 | <0.001 |
| Mg$^{2+}$               | 2  | 2.592| 0.828| 375.980 | <0.001 |
| Ca$^{2+}$:CO$_3^{2-}$   | 1  | 0.510| 0.510| 148.143 | <0.001 |
| Mg$^{2+}$ * Ca$^{2+}$:CO$_3^{2-}$ | 2  | 1.040| 0.520| 150.924 | <0.001 |
| Error                   | 24 | 0.083| 0.003|         |        |
| Total Error             | 29 | 4.224|      |         |        |

3.2. Mineralogy and Crystal Morphology

Back-scatter electron (BSE) images of the CaCO$_3$ surfaces show distinct morphological differences between the six treatments. The Mg-free (control Mg treatment) 47:1 Ca:CO$_3$ incubations formed homogeneous and heterogeneous nucleation in the form of crosshatched vaterite pre-spherical and laminated cubed calcite (Figure 4a). In the Mg-free 2.8:1 scenario, laminated cube calcite precipitated, along with amorphous calcium carbonate (ACC) or a stable prenucleation calcium carbonate cluster c.f. [47], and presumably unfinished calcite transforming from proto-vaterite precursors (Figure 4b). Despite the absence of a measurable weight increase from both the Mg addition scenarios with the 2.8:1 Ca:CO$_3$, newly formed aragonite needles were visibly precipitated on top of the seeding material (Figure 4d,f). Well-defined and abundant acicular crystals were precipitated in random directions and from multiple centers of nucleation as well as from cemented CaCO$_3$ (Figure 4f). In the scenario with high Mg concentration (equal to present day seawater, 53 mM) and at a 2.8:1 Ca:CO$_3$, cements were also observed along with dissolution pits in a needle form (Figure 4f). In the scenario with lower Mg concentration (equal to half the present-day seawater, 26.5 mM) with a 47:1 Ca:CO$_3$, both ACC and dissolution pits were observed, in addition to Mg-calcite (Figure 4c). Conversely, in the scenario with the high Mg concentration and 47:1 Ca:CO$_3$, cements primarily formed along with dissolution pits, and low-relief aragonite needles within the seed material crevices (Figure 4e).
4. Discussion

This study compartmentalizes hypothetical abiotic conditions of the ECF, with the aim to gain a broader understanding of the chemical mechanisms relating to biomineralization among tropical marine calcifiers. We show that despite the same ion product of Ca and CO\textsubscript{3}, calcification rates vary with different Ca:CO\textsubscript{3} ratios and Mg concentrations. In agreement with [31,36], this study emphasizes the importance of considering the ratio of Ca:CO\textsubscript{3} when estimating the $\Omega_{\text{calcite}}$ within the ECF of marine calcifiers as exemplified in Figure 1. It is worth noting that in these experiments calcite and vaterite were also precipitated. The calculated $\Omega_{\text{vaterite}}$ in all experiments was 10, $\Omega_{\text{calcite}}$ was 15.15. Even though $\Omega_{\text{vaterite}}$ has been reported to be lower than $\Omega_{\text{ara}}$ and $\Omega_{\text{calcite}}$ it is not possible to calculate $\Omega_{\text{vaterite}}$ because this requires knowledge of the solubility product [49,50]. Our results show that high calcification rates, are not possible when the Mg concentration is equal to or half that of present-day oceanic concentrations, unless it is counterbalanced by a number of additional factors such as Ca:CO\textsubscript{3} stoichiometry, temperature, $\Omega_{\text{ara}}$, proton pumping, or organic molecules. This study infers that the specific conditions required for CaCO\textsubscript{3} precipitation among marine
calcifiers is positively amplified by the organism. It is difficult to fully understand the process that controls biomineralization without further in situ ionic measurements from the ECF or more in vitro experiments.

4.1. Comparing Low and High Ca:CO$_3$ Scenarios

Generalized CaCO$_3$ precipitation models, as described by [23,28], present disagreement regarding the values for the coefficients n and k [24,25,51], which overestimate the calcification flux at low carbonate concentrations, e.g., when the ECF becomes DIC limited. It is well documented that biomineralization requires elevated Ω and in doing so implies DIC concentrating mechanisms [28,44–46]. However, direct DIC measurements from the ECF within tropical corals indicate concentrations similar to that of ambient seawater [37], which may result from high DIC consumption during calcification. As seen in previous studies, calcification among tropical corals can be maintained by elevating the Ca ions in the ECF to compensate decreasing seawater pH [43], while decreasing the strontium:calcium [Sr:Ca] and borate [B(OH)$_4$] ratios [52]. When comparing the low Ca:CO$_3$ (mimicking strong proton removal from the ECF resulting in elevated $T_A$ four times greater than ambient seawater, and DIC three times greater than ambient seawater) with the high Ca:CO$_3$ scenario [ambient seawater DIC and $T_A$] at elevated pH = 8.7 and Ω$_{ara}$ = 10 and 0 Mg [control Mg treatment], calcite precipitation rates were three times greater in the ambient seawater treatment than in the DIC concentrating mechanism treatment (Figure 3). This implies that ambient DIC is sufficient to induce calcification provided that homeostasis is maintained in the ECF and the DIC withdrawal from calcification is balanced by ionic flow rates in and out of the ECF [44–46].

4.2. The Connection between Mg and Calcification

Marine carbonate-producing organisms exert strong biogenic control to promote calcification within their ECF. This biogenic control is evident by the various mineralogy types and microstructures found among marine carbonate-producing organisms [53]. Calcite is preferentially precipitated as a function of lower temperatures and/or Mg:Ca ratios [54], in addition to the preferential substitution of Ca for Mg, e.g., high-Mg calcite [25]. For example, previous studies have shown coralline algae [33], scleractinian corals [34], and juvenile scleractinian coral [55] can produce calcite when the Mg:Ca ratio of seawater is <2 (e.g., Cretaceous calcitic seas) but at a slower rate. A recent study also found the presence of Mg ions to inhibit not only calcite nucleation during crystal formation but also aragonite [56]. Similarly, it has been shown that strontium also inhibits precipitation rates as a direct correlation with the aqueous calcium activity, thus preventing the attachment of calcium ions to the reactive sites [57,58]. Aragonite microstructure has also been shown to vary as a function of calcification rate, from rapidly formed granular centers of calcification to slower formed fibrous needles [59,60] as determined by the fractionation of $\delta^{18}$O and $\delta^{13}$C isotopes [61,62] and Mg:Ca ratios [59,60,63].

4.3. Polynucleation and Spontaneous Nucleation

The ratio of calcium to carbonate clearly matters within the ECF, as it has been shown to describe the rate and morphology of CaCO$_3$ (Figures 3 and 4). Precipitation pathways can be either direct or sequential depending on the free energy available on the surface as determined by pre-nucleation clusters (PNC), growth, and transformation [64–67]. Polynucleation occurred in all scenarios in this study, which led to a complex situation increasing the number of active sites on the surface layer, and therefore a stronger dependence on supersaturation than solely the layer-by-layer mineralization process [31]. Further complications arise because the rate-determining step may change with time as the number of defects and the relative dimensions of the crystal faces become modified during precipitation. Therefore, there are often deviations from the idealized kinetic models, as there may be a number of mechanisms operating in concert [66–68]. It is interesting to note
that spontaneous nucleation appeared in the experiment with high excess of calcium ions relative to carbonate ions (i.e., the ambient DIC and TA seawater treatment) but not in the low Ca:CO$_3$ treatment (i.e., the DIC concentrating mechanism scenario), however previous studies have shown that PNC usually form in a low Ca:CO$_3$ solution, equivalent to the binding of ions during crystal formation [47]. This may be due to metastable conditions under which precipitation of the mineral is delayed despite the solution being oversaturated in respect to $\Omega_{\text{ara}}$ [67].

The higher calcification rates in the Mg-free and 47:1 Ca:CO$_3$ scenario were obtained primarily due to spontaneous nucleation within the incubation chamber leading to much higher precipitation and thus greater reactive surface area. Previous calcite precipitation experiments in supersaturated ($\Omega_{\text{calcite}}$ 5, 16; pH = 10; T = 20 °C) conditions did not produce spontaneous nucleation and showed an optimum precipitation rate when Ca:CO$_3$ = 1:1 [31]. However, there are differences between this study and [31], one of which is the use of NaHCO$_3$ to prepare the carbonate solution in this study instead of K$_2$CO$_3$. This together with a temperature difference of 5 °C can potentially explain the variation between our observations and [31].

4.4. CaCO$_3$ Polymorphs

In the SEM images (Figure 4), the Mg-free high Ca:CO$_3$ scenario we see ACC, metastable inter-crosshatched vaterite pre-spheres, and rhombohedral calcite blocks. The sequential dissolution and re-precipitation mechanism can be explained via the kinetic rate, which is primarily controlled by the surface area of the crystal [69]. The mixture of vaterite and calcite suggests that calcite mineralization is the rate-determining step. The substitution of Mg into the ACC will however precipitate directly into calcite without the intermediate vaterite phase [70,71] as seen in the 2.5 Mg high Ca:CO$_3$ scenario (Figure 4c). Under the present-day Mg:Ca ratio, aragonite dominates the kinetics of nucleation due to the calcite nucleation barrier being greater than metastable aragonite [72], which explains the lack of calcite in the high-Mg scenario. However, nucleation and precipitation in both the high-Mg scenarios where close to zero, implying that the aragonite seeding material was in equilibrium with the solution as shown by the dominance of ACC (Figure 4d–f) and dissolution pits in the shape of aragonite needles (Figure 4f).

The Ca:CO$_3$ ratio as well as the Mg concentration affected the CaCO$_3$ polymorph precipitated from the oversaturated solutions (Figure 4). In the Mg-free incubations, we obtained crosshatched vaterite and layered rhombohedral calcite in the high Ca:CO$_3$ scenario (Figure 4a) and an intermediate form of ACC together with rhombohedral calcite in the low Ca:CO$_3$ scenario (Figure 4b). In the incubations with 26.5 mM Mg, we obtained an unconnected Mg-calcite in the high Ca:CO$_3$ scenario (Figure 4c) and aragonite needles in the low Ca:CO$_3$ scenario (Figure 4d). With a 53 mM Mg concentration, representing normal seawater conditions, very little new material precipitated, most of which were ACC with sparse low relief aragonite needles (Figure 4e) or unconnected aragonite needles with dissolution pits in the form of needles (Figure 4f). Varying the Ca:CO$_3$, while keeping the Mg concentration fixed, changes the Mg:Ca ratio, which may have driven the differences in polymorphs shown in Figure 4c,d. Overall, the variety of polymorphs precipitated at a pH of 8.7 and an $\Omega_{\text{ara}}$ of 10 demonstrates that $\Omega$ alone does not control the precipitation process, as also suggested by [73]. Therefore, caution should be applied when inferring saturation state from the crystal morphology [60], particularly if other factors, e.g., Mg concentrations, temperature, or DIC, are not known.

Even though this study removes the organic aspect of biomineralization, organic molecules have been shown to act as a template to facilitate or induce crystallization [20,74–77] due to their strong binding potential with calcium ions [78,79]. The source of the organics is likely a combination of polyp-derived SOM and seawater-derived SOM as demonstrated from a comparison of coral skeletons and abiotic aragonite [80]. However, the presence of SOM or coral mucus in oversaturated solutions has also been shown to inhibit the nucleation of CaCO$_3$ [81] or pose no effect towards the rate of calcification [31]. Rather,
organic molecules appear to influence the CaCO$_3$ polymorph that precipitates from an oversaturated solution [31,77,82]. This suggests that organic molecules have a greater influence on the processes at the crystal surface that leads to the formation of a crystal structure, but not the kinetic processes, which transports the ions to the crystal surface.

An interesting observation from this study is that the aragonite needles precipitated in synthetic seawater (observed in the 26.5 mM Mg with a 2.8:1 Ca:CO$_3$ treatment) with no added biomolecules have a similar morphological appearance to synthetic aragonite experiments made from natural seawater, presumably with some residual organic carbon [60]. This could suggest that coral aragonite crystals may precipitate abiogenically after being initially nucleated, since abiotic systems that lack biomolecule templates altogether show similar morphologies.

4.5. Implications for Coral Reef Calcifiers

The concentrations of Mg or PO$_4$, which actively influence crystallization [21,23,24,67,83], are not well known for the ECF among marine organisms. Several studies assume the Mg concentrations in the ECF to be the same as in seawater and thus imply very high $\Omega_{\text{ara}}$ (>20) in order to explain the high precipitation rates as observed in corals [11,23,24,70–79,81,84]. Pioneer studies, which utilized various techniques, are largely in agreement with the range of $\Omega_{\text{ara}}$ in the ECF. For example, based on microsensor measurements, $\Omega_{\text{ara}}$ ranges from 11–25.5 [11,12,38,46], with the exception of 3.2 in the dark [11], 11–12.3 from Raman spectra [43,85], 11–25 inferred from $\delta^{11}$B isotopes [44,86], 11.1–17.3 predicted by X-ray diffraction-based crystallographic estimates [87], and previously reviewed by [38] to range between 10.16–38.31. While, previous studies have reported the pH in the ECF to be 0.5–0.2 units higher than ambient seawater [46] and that homeostasis can be maintained within the ECF regardless of varying external seawater pH [88]. Thus, implying a wide range of plausible Ca:CO$_3$ and Ca:Mg ratios within the ECF which enable CaCO$_3$ precipitation.

Measured Mg:Ca ratios from coral skeletons are between 1.5–5.5 mmol/mol [89–92] and from inorganic aragonite has between ~8.5–10 mmol/mol [59], while inorganic calcite has between 30–140 mmol/mol [93], demonstrating the importance of a Mg removal mechanism to facilitate the rate and morphology of calcification. Our results show that high calcification rates observed in corals are not possible when the Mg ion concentration in the ECF is equal to or half that of present day oceanic concentrations (Mg:Ca > 2.5). The high calcification rates observed in this study suggest a mechanism for active removal of inhibiting ions such as Mg from the coral ECF or a Ca concentration mechanism as suggested previously [43]. These points stress the well held belief that biomineralization is a highly complex and biologically mediated process, orchestrated by the secretion of organic molecules [94] and active ion transport [29].

Heterogeneous nucleation is largely inferred by the presence of biomolecules such as acid-rich proteins (e.g., sulphated proteoglycans) and various adhesion and structural proteins [10,18,19] are considered vital for the promotion and functioning of CaCO$_3$ structures. Additionally, the presence of SOM is known to influence the CaCO$_3$ crystal polymorph precipitated from over saturated solutions [82]. Recent experiments [36] confirmed an inhibiting role of coral organic molecules towards rate but not form of CaCO$_3$ [81]. For instance, the role of an ACC precursor phase is likely initiated by a series of controlled biomineralization mechanisms [95,96], particularly for polymorphic calcifying marine organisms. Heterogeneous nucleation has been observed in a range of marine calcifiers such as barnacles [97], echinoderms [95,98], coralline algae [99], foraminifera [100], and corals [43,96].

5. Conclusions

To understand the nuances of how coral reef calcifiers can adapt to global change, such as ocean acidification, we need to better understand the ionic composition at the site of calcification. Unfortunately, in the short-term tropical calcifying organisms show little acclimatization potential to ocean acidification [101] particularly when coupled with thermal
stress [102], but there are few examples of resistance by altering the ionic concentrations in the ECF, for example Ca [43]. Additionally, this study considered the influence of various Ca:CO$_3$ stoichiometry and Mg concentrations on the precipitation rates and morphology of CaCO$_3$ in a homeostatic experiment. Although there is still a need to conduct more experiments covering a range of other possible scenarios, we believe that our findings are highly relevant within the field of coral reef research for the following reasons:

1. Varying concentrations of calcium and carbonate ions at fixed $\Omega_{\text{ara}}$ demonstrates the underlining principal that calcium and carbonate ion concentrations can obtain the same $\Omega_{\text{ara}}$ value at different Ca:CO$_3$ stoichiometry and questions the generalized applicability of the empirical equation that prescribes the calcification rate as a function of $\Omega_{\text{ara}}$ alone.

2. As shown, calcifying fluid stoichiometry alters the precipitation rate and morphology of CaCO$_3$ at a constant $\Omega$ and pH. Therefore, our findings suggest caution should be applied when inferring saturation state from the crystal morphology, particularly if other factors e.g., Mg, temperature, or DIC are not known.

3. When comparing a strong proton removal scenario and a DIC concentrating mechanism to a scenario with ambient seawater pH and DIC conditions, calcite precipitation rates were three times greater in the ambient seawater conditions. Implying ambient seawater pH and DIC within the calcifying fluid is sufficient to induce calcification provided homeostasis is maintained.

4. Mg exerts a stronger effect on the instability of CaCO$_3$ than Ca:CO$_3$ stoichiometry, in which Mg incorporation locally disturbs the coordination environment in the aragonite structure [87,103]. These differences emphasize the importance of Mg removal from the calcifying fluid. Future studies are recommended to additionally monitor the Mg concentration in the calcifying fluid along with the carbon chemistry.

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Appendix A

Table A1. Initial and final seed weights, including the duration of the experiment and the standardized weight change. Experimental identification corresponds to the aqueous conditions detailed in Table 1. The seed weight values are listed in grams, the experimental identification code is listed as Exp., the individual CaCO$_3$ bioclast identification is listed under Seed, the initial weight and end weight of the seed material correspond to column $t_0$ and $t_e$, the number of days in the experimental conditions is in column D (variation in experimental duration was due to unexpected health and safety issues), and the standardized weight change per day (g d$^{-1}$) is in column D$\Delta$.

| Exp. | Seed       | $t_0$ | $t_e$ | D  | D$\Delta$ |
|------|------------|-------|-------|----|-----------|
| 1a   | 1Mg-Free   | 0.480 | 30.290| 32 | 0.932     |
| 1a   | 2Mg-Free   | 0.657 | 36.500| 32 | 1.120     |
| 1a   | 3Mg-Free   | 0.883 | 30.620| 32 | 0.929     |
| 1a   | 4Mg-Free   | 1.057 | 30.220| 32 | 0.911     |
| 1a   | 5Mg-Free   | 1.202 | 39.370| 32 | 1.193     |
| 1b   | 6Mg-Free   | 0.591 | 9.320 | 32 | 0.273     |
| 1b   | 7Mg-Free   | 0.862 | 5.260 | 32 | 0.137     |
| 1b   | 8Mg-Free   | 0.983 | 7.480 | 32 | 0.203     |
| 1b   | 9Mg-Free   | 1.064 | 10.300| 32 | 0.289     |
| 1b   | 10Mg-Free  | 1.218 | 9.070 | 32 | 0.245     |
| 2a   | 1Mg        | 0.212 | 0.310 | 70 | 0.001     |
| 2a   | 2Mg        | 0.715 | 0.570 | 70 | −0.002    |
| 2a   | 3Mg        | 0.777 | 0.649 | 70 | −0.002    |
| 2a   | 4Mg        | 0.924 | 0.718 | 70 | −0.003    |
| 2a   | 5Mg        | 1.013 | 1.006 | 70 | 0.000     |
| 2b   | 6Mg        | 0.423 | 0.322 | 70 | −0.001    |
| 2b   | 7Mg        | 0.766 | 13.003| 70 | 0.175     |
| 2b   | 8Mg        | 0.818 | 0.793 | 70 | 0.000     |
| 2b   | 9Mg        | 0.972 | 0.944 | 70 | 0.000     |
| 2b   | 10Mg       | 1.336 | 1.381 | 70 | 0.001     |
| 3a   | 1Mg +      | 0.364 | 0.360 | 38 | 0.000     |
| 3a   | 2Mg +      | 0.816 | 0.643 | 38 | −0.005    |
| 3a   | 3Mg +      | 0.947 | 0.902 | 38 | −0.001    |
| 3a   | 4Mg +      | 1.150 | 1.146 | 38 | 0.000     |
| 3a   | 5Mg +      | 1.274 | 1.212 | 38 | −0.002    |
| 3b   | 6Mg +      | 0.668 | 0.534 | 38 | −0.004    |
| 3b   | 7Mg +      | 0.918 | 0.922 | 38 | 0.000     |
| 3b   | 8Mg +      | 1.069 | 1.064 | 38 | 0.000     |
| 3b   | 9Mg +      | 1.220 | 1.237 | 38 | 0.000     |
| 3b   | 10Mg +     | 1.449 | 1.485 | 38 | 0.001     |

Table A2. The quantity of compounds (mg/5 L) added to the stock solution to obtain the experimental parameters outlined in Table 1.

| Exp. | Calcium Stock (mg/5 L) | Carbonate Stock (mg/5 L) |
|------|------------------------|--------------------------|
|      | CaCl$_2$ | MgCl$_2$ | NaCl | NaHCO$_3$ | NaCl |
| 1a   | 15.583  | 0.000    | 164.416 | 1.493    | 178.507 |
| 1b   | 3.809   | 0.000    | 176.191 | 6.107    | 173.893 |
| 2a   | 15.583  | 53.874   | 110.542 | 1.493    | 178.507 |
| 2b   | 3.809   | 53.874   | 122.316 | 6.107    | 173.893 |
| 3a   | 15.583  | 107.749  | 56.667  | 1.493    | 178.507 |
| 3b   | 3.809   | 107.749  | 68.442  | 6.107    | 173.893 |
Figure A1. CaCO$_3$ polymorphs (aragonite, calcite, and vaterite) were identified via Raman spectroscopy, which were first morphologically identified by SEM on the incubated crystals. These Raman spectra were used to qualitatively identify the crystal structures found on the seeding crystals after incubation.

Script A1. Below are the calculations used to modify the artificial seawater for the six experiments.

```python
# script for calculating salts and acids to set up solutions for experiment
import numpy as np
import scipy.optimize as opt

# some functions

def KstarW(tempK, salt):  # after Millero_1995 p.670 Eq.63 and OA best practices guide
    a0 = -1.384726e4
    a1 = 1.489652e2
    a2 = -2.36521e1
    b0 = 1.1867e2
    b1 = -5.977
    b2 = 1.0495
    g = -1.615e-2
    lnKWT = a0/tempK + a1 + a2*np.log(tempK)
    fT = b0/tempK + b1 + b2*np.log(tempK)
    kstarw = np.exp(lnKWT + fT*(salt**0.5) + g*salt)
    return kstarw

def Kstar1(tempK, salt):  # after OA best practices guide
    kstar1 = 10**(−3633.86/tempK + 61.2172 −9.67770*np.log(tempK) + 0.011555*salt −0.0001152*salt**2.)
    return kstar1

# some functions

def Kstar2(tempK, salt):  # after OA best practices guide
    kstar2 = 10**(−471.78/tempK −25.9290 + 3.16967*np.log(tempK) + 0.01781*salt −0.0001122*salt**2.)
    return kstar2

def Ksp_ara(tempK, salt):
    ksp_ara = -171.945−0.077993*tempK + 2903.293/tempK + 71.595*np.log10(tempK) + (-0.068393 +0.0017276*tempK + 88.135/tempK)*salt**0.5−0.10018*salt + 0.0059415*salt**1.5 #mol2 kg−2
    return 10.0**ksp_ara

def Kstar0(tempK, salt):  # after Weiss, R. F., Marine Chemistry 2:203−215, 1974. (taken from CO2sys)
    TempK100 = tempK/100.0;
```

---

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\[
\ln K_0 = -60.2409 + 93.4517/\text{TempK100} + 23.3585 \times \log(\text{TempK100}) + \text{salt} \times (0.023517 - 0.023656 \times \text{TempK100} + 0.0047036 \times \text{TempK100}^2).
\]

\[
K_0 = \exp(\ln K_0) \quad \text{# this is in mol/kg-SW/atm}
\]

return K0

def Ksp_cal(tempK, salt):
    ksp_cal = -171.9065 - 0.077993*tempK + 2839.319/tempK + 71.595*log(tempK) + (-0.77712 + 0.0028426*tempK + 178.34/tempK)*salt**0.5 - 0.07711*salt + 0.0041249*salt**1.5 # mol2 kg−2

return 10.0**ksp_cal

# molar masses
m_Na = 22.98977 # [g/mol]
m_Ca = 40.078 # [g/mol]
m_Mg = 24.3050 # [g/mol]
m_Cl = 35.4527 # [g/mol]
m_C = 12.0107 # [g/mol]
m_O = 15.9994 # [g/mol]
m_H = 1.00794
m_H2O = 2*m_H + m_O
m_CaCl2 = m_Ca + 2.0*m_Cl + 2.0*m_H2O # [g/mol]
m_MgCl2 = 203.30 # [g/mol]
m_NaCl = m_Na + m_Cl # [g/mol]
m_Na2CO3 = 2.0*m_Na + m_C + 3.0*m_O # [g/mol]
m_NaHCO3 = m_Na + m_H + m_C + 3*m_O

# constant forcing and salt matrix
Temperature = 25.0 # Celsius
Salinity = 36.0 # we use g per kg
TK = 273.15 + Temperature
Sal = Salinity
K0F = Kstar0(TK, Sal)
K1F = Kstar1(TK, Sal)
K2F = Kstar2(TK, Sal)
KWF = KstarW(TK, Sal)
Ksp = Ksp_ara(TK, Sal)
Ksp_cal = Ksp_cal(TK, Sal)

# input variables:
# target values (in the experiment):
PH_chamber = 8.7
Omega_chamber = 10.0
Mg_chamber = 53.0e−3/1.0 # mol kg−1
Ca_chamber = 10.60e−3 # mol kg−1
# or
Stoichiometry = 1.0/1.0 # mol Ca : mol CO3
Ca_chamber = np.sqrt(Omega_chamber*Ksp*Stoichiometry)
H_chamber = 10.0**(-PH_chamber)

# calculated values
# for the calculation of calcium and carbonate ions, I use two equations:
# Stoichiometry = Calcium / CO3
# Omega = Calcium * CO3 / Ksp
# now I solve for CO3
# $\text{CO}_3$ = Calcium/Stoichiometry

# $\text{CO}_3$ = Omega*$K_{sp}$/Calcium
# Calcium/Stoichiometry = Omega*$K_{sp}$/Calcium * Calcium * Stoichiometry
# Calcium**2 = Omega*$K_{sp}$*Stoichiometry

# once I know Calcium the rest is as follows
# (Chamber values)

$\text{CO}_3_{\text{chamber}}$ = Omega$_{\text{chamber}}$*$K_{sp}$/Ca$_{\text{chamber}}$ mol kg$^{-1}$

$\text{DIC}_{\text{chamber}}$ = CO$_3_{\text{chamber}}$(K1F*H$_{\text{chamber}}$ + H$_{\text{chamber}}$*H$_{\text{chamber}}$ + K1F*K2F)/(K1F*K2F)

$\text{CAlk}_{\text{chamber}}$ = DIC$_{\text{chamber}}$*K1F*(H$_{\text{chamber}}$ + 2.0*K2F)/(H$_{\text{chamber}}$*H$_{\text{chamber}}$ + K1F*H$_{\text{chamber}}$ + K1F*K2F)

OH$_{\text{chamber}}$ = KWF/H$_{\text{chamber}}$

TA$_{\text{chamber}}$ = CAlk$_{\text{chamber}}$ + OH$_{\text{chamber}}$ - H$_{\text{chamber}}$

Omegacal = Ca$_{\text{chamber}}$*CO$_3_{\text{chamber}}$/$K_{sp_{\text{cal}}}$

print 'Omega calcite = ', Omegacal

#************************************************************************************************************

# output
# (Chamber values)

print 'expected values:'
print 'Ca =', Ca$_{\text{chamber}}$*1e3, 'e$^{-3}$ mol kg$^{-1}$'
print 'Mg =', Mg$_{\text{chamber}}$*1e3, 'e$^{-3}$ mol kg$^{-1}$'
print 'CO3 =', CO$_3_{\text{chamber}}$*1e6, 'e$^{-6}$ mol kg$^{-1}$'
print 'pH =', pH$_{\text{chamber}}$
print 'DIC =', DIC$_{\text{chamber}}$*1e6, 'e$^{-6}$ mol kg$^{-1}$'
print 'TA =', TA$_{\text{chamber}}$*1e6, 'e$^{-6}$ mol kg$^{-1}$'
print 'Omega =', Omega$_{\text{chamber}}$
print 'stoichiometry =', Ca$_{\text{chamber}}$/CO$_3_{\text{chamber}}$, (mol Ca : mol CO3)
print 'Cai paper: Omega=', 10.6e$^{-3}$*600.0e$^{-6}$/Ksp

#************************************************************************************************************

# (Bag values)

print 'the amounts of salts needed are:'

$g_{\text{CaCl}_2}$ = Ca$_{\text{chamber}}$*m$_{\text{CaCl}_2}$*2.0 # times two because the concentrations will be diluted in the chamber

$g_{\text{MgCl}_2}$ = Mg$_{\text{chamber}}$*m$_{\text{MgCl}_2}$*2.0 # times two because the concentrations will be diluted in the chamber

print $g_{\text{CaCl}_2}$*5.0, 'g CaCl2 per 5 Liters'

print $g_{\text{MgCl}_2}$*5.0, 'g MgCl2 per 5 Liters'

# (Bag values)

$g_{\text{NaHCO}_3}$ = DIC$_{\text{chamber}}$*m$_{\text{NaHCO}_3}$*2.0# times two because the concentrations will be diluted in the chamber

print $g_{\text{NaHCO}_3}$*5.0, 'g NaHCO3 per 5 Liters'

print 'and to adjust salinity in the solutions we need:'

$g_{\text{NaCl}_1}$ = Salinity-$g_{\text{CaCl}_2}$-$g_{\text{MgCl}_2}$ # since Salinity is defined as g/kg

print $g_{\text{NaCl}_1}$*5.0, 'g NaCl per 5 Liters in the CaCl2 bag:'

$g_{\text{NaCl}_2}$ = Salinity-$g_{\text{NaHCO}_3}$ # since Salinity is defined as g/kg

print $g_{\text{NaCl}_2}$*5.0, 'g NaCl per 5 Liters in the NaHCO3 bag:'

print 'remark:'
print 'Although I believe that the amount of carbonate might be overestimated and that measured Salinity might actually be lower. However, we can test this with a calibrated salinity electrode.'

#************************************************************************************************************

print ''
print ''
print 'and the pH of the solutions will be:'
C0 = DIC$_{\text{chamber}}$*2.0
print 'DIC = ', C0
pKW = -np.log10(KWF)
\[
pKS1 = -\log_{10}(K1F)
pKS2 = -\log_{10}(K2F)
pKB1 = \text{pK}_W - pKS1
pKB2 = \text{pK}_W - pKS2
\]

\[
pH_{NaHCO3} = \frac{1}{2}(pKS1 - \log_{10}(C0))
\]

# this does not work because the approximation that the acid is only a 1 proton acid is too crude.

\[
pH_{NaHCO3} = \text{pK}_W - 0.5(pKB1 - \log_{10}(C0))
\]

# for an amphoter

\[
pH_{NaHCO3} = \frac{1}{2}(pKS1 + pKS2)
\]

# the function of the H+ concentration is an equation of fourth order and has to be solved numerically
# I use fmin to solve it:

\[
\text{DIC}_{CO3} = \text{DIC}_{chamber} \times 2.0
\]

# the equation differs if you use NaHCO3 because the charge balance is slightly different:

\[
\text{H}_\text{func}_{NaHCO3}(H): \quad \text{val} = (\text{KW}/H + H*\text{DIC}_{CO3}/(H*H/(K2*K1) + H/K2 + 1.0)/K2 + 2.0*\text{DIC}_{CO3}/(H*H/(K2*K1) + H/K2 + 1.0) - H - \text{DIC}_{CO3}^2)
\]

# we will not adjust the pH of the CaCl2 bag !!!
# the milliQ is cooked and has a pH of 7
# adding CaCl2 does not add alkalinity, it might have a small effect on the pH due to CaOH and CaOH2
# we assume TA_Ca = 0.0; DIC_Ca = 0.0; and pH_Ca = 7.0
# (Bag values) Calcium Bag:

\[
\text{DIC}_{Ca} = 0.0
\]

# now I have to calculate the required TA of the CO3 bag, which is double the TA in the chamber

\[
\text{TA}_{CO3} = \text{TA}_{chamber} \times 2.0
\]

# this is the amount of alkalinity that has to be added via NaOH
# so, basically, I have the amount of NaOH that has to be added (at least in theory)
print 'diff TA =', diff_TA*1e6
# (Bag values) Carbonate Bag:
#H_CO3 = 10.0^(-pH_target)
#OH_CO3 = KWF/H_CO3
#CAlk_CO3 = DIC_CO3*K1F*(H_CO3 + 2.0*K2F)/(H_CO3*H_CO3 + K1F*H_CO3 + K1F*K2F)
#TA_CO3 = CAlk_CO3 + OH_CO3 - H_CO3
#0 = DIC_CO3*K1F*(H_CO3 + 2.0*K2F)/(H_CO3*H_CO3 + K1F*H_CO3 + K1F*K2F) + KWF/H_CO3 - H_CO3 - TA_CO3
# the equation differs if you use NaHCO3 because the charge balance is slightly different:
def H_func_CO3bag(H):
    val = (TA_CO3 - (DIC_CO3*K1F*(H + 2.0*K2F)/(H*H + K1F*H + K1F*K2F) + KWF/H - H))**2.0
    return val
# take a good guess from the approximation
H_init = 10**(-pH_chamber)
H_opt = opt.fmin(H_func_CO3bag, H_init, xtol = 1e-12, ftol = 1e-12, maxiter=None, maxfun=None, full_output=0, disp=1, retall=0, callback=None)
print 'print target H for the NaHCO3 bag:'
print 'H for NaHCO3=', H_opt
pH_CO3_opt=-np.log10(H_opt)
print 'print target pH for the NaHCO3 bag:'
print 'the resulting pH of the NaHCO3 solution is', pH_CO3_opt
# test if this pH results the correct TA
CAlk_opt = DIC_CO3*K1F*(H_opt + 2.0*K2F)/(H_opt*H_opt + K1F*H_opt + K1F*K2F)
OH_opt = KWF/H_opt
TA_opt = CAlk_opt + OH_opt - H_opt
print 'TA CO3', TA_CO3*1e6, 'minus TA opt', TA_opt*1e6, '=', (TA_CO3-TA_opt)*1e6
#************************************************************************************************************
# now titrate the NaHCO3 bag to the target pH
# and the same for the other solution
# (Bag values)
pH_start = 7.968 #pH_NaHCO3_opt
pH_target= pH_CO3_opt
#amount of acid needed (first calculated without buffering capacity)
mol_NaOH=10.0**(-pH_start) - 10.0**(-pH_target)
# I have to calculate the amount of protons that are consumed also by the buffering system of the carbonate chemistry,
# and this on top of the pH change without the buffering capacity.
H_start=10.0**(-pH_start)
H_end=10.0**(-pH_target)
OH_start=KWF/H_start
OH_end=KWF/H_end
CO3_start=DIC_CO3*K1F*K2F/(K1F*H_start + H_start*H_start + K1F*K2F)
HCO3_start=DIC_CO3*K1F*H_start/(K1F*H_start + H_start*H_start + K1F*K2F)
H2CO3_start=DIC_CO3*HCO3_start-CO3_start
CO3_end=DIC_CO3*K1F*K2F/(K1F*H_end + H_end*H_end + K1F*K2F)
HCO3_end=DIC_CO3*K1F*H_end/(K1F*H_end + H_end*H_end + K1F*K2F)
H2CO3_end=DIC_CO3*HCO3_end-CO3_end
print 'CO3 from:', CO3_start*1e6, 'to', CO3_end*1e6, '\mu mol'
print 'HCO3 from:', HCO3_start*1e6, 'to', HCO3_end*1e6, '\mu mol'
print 'H2CO3 from:', H2CO3_start*1e6, 'to', H2CO3_end*1e6, '\mu mol'
H_diff=(2*H2CO3_start+HCO3_start+H_start*OH_start)-(2*H2CO3_end+HCO3_end+H_end*OH_end)
print 'mol NaOH needed to adjust pH_NaHCO3 from', pH_start, 'to', pH_target, 'is:', H_diff, 'compared to:', mol_NaOH, 'without considering the buffer capacity'
print 'at a molarity of', mol_base, 'this requires ', H_diff/mol_base*1e3*5.0, 'ml of base for 5 L solution'

# added for review:
# calculate Omega calcite for comparison
Omega_cal=Ca_chamber*CO3_chamber/Ksp_cal
print 'Omega calcite=', Omega_cal
Omega_araga=Ca_chamber*CO3_chamber/Ksp
print 'Omega aragonite=', Omega_araga

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