

Dear Editor,

Thank you for providing us the opportunity to revise our manuscript, “Coral calcifying fluid aragonite saturation states derived from Raman spectroscopy”. Comments from the two reviewers highlighted several areas of the manuscript where additional details and explanations were required. We are confident that in revising according to the reviewers’ suggestions that we improved the quality of our manuscript. Below are responses to each comment, including specific references to the changes we made in the manuscript.

Reviewer 1:

Comment 1: The submitted manuscript of DeCarlo et al. reports interesting innovative research results regarding the use of Raman spectroscopy for the determination of aragonite saturation state in inorganic experiments and during coral calcification. As such it is timely, of broad scientific interest and fits into the scope of BG. I would expect this material to be publishable after careful revision.

Before explaining some of my concerns I need to underline I am not at all a Raman spectroscopy expert. Thus, Raman-specific technical details need to be reviewed by a respective expert before any decision on publication can be made.

In general I like this manuscript for its interesting approach which warrants publication in my opinion. Nevertheless, some conclusions, I think, should be presented more carefully, underlining the potential and open questions of this approach.

***Response 1:* We thank the reviewer for the supportive comments and careful consideration of our manuscript. We appreciate the issues raised, which highlight a couple areas of the manuscript that require clarification. Below, we respond to the reviewer’s specific comments. While our results and conclusions remain the same, we agree with the reviewer that we needed to add a few details to further explain how the calculations were performed.**

Comment 2: It appears strange to me, the calibration (inorganic) yields results for the coral which are presented as correct reconstruction of internal ω saturation state. The calibration in my opinion is not entirely correct or it is not very clearly explained. I’d tried to recalculate the regression based on the data provided in the supplements. It appears, the uncertainties of the saturation state data from the experiment have not been included in the uncertainty estimate of the calibration. It rather looks like the means of FWHM plotted vs. Ω and a log-fit applied. This is critical as later the FWHM is used to reconstruct Ω . I hope, the point is clear, it would need quite the opposite plot and fit, Ω vs. FWHM for a calibration useful for the desired calculation. Well, the fit obviously, changes in this case.

Response 2: We thank the reviewer for this comment, which alerted us to a point that we needed clarification. The reviewer is correct that our calibration is based on fitting FWHM to the log of Ω_{Ar} (see Table 2). We use Ω_{Ar} as the independent variable (x-axis in Figure 2) and FWHM as the dependent variable (y-axis in Figure 2) because our proposed mechanism is that Ω_{Ar} causes changes in FWHM (see section 4.1). However, we agree with the reviewer that this type of regression does not take errors in Ω_{Ar} into account, and that an alternative approach would be to fit Ω_{Ar} as a function of FWHM. We tried this method using the measured data from the WITec instrument (Figure R1-1 below, new Figure S6, and page 7 lines 27-28). The difference between the two approaches is very small, *e.g.* approximately 0.1 Ω_{Ar} unit difference for JCp-1.

Comment 3: In any way, I could not replicate the Jcp-1 Omega of 12.3 with either of the calibrations. Could it be, each individual Raman result had been converted into a result for Omega and those results have been used to calculate an average of 12.3? If so, I did not get this from the manuscript... The Omega calculated from the mean FWHM would be >13, if I did the calculations right.

Response 3: While we cannot reproduce the Ω_{Ar} of 13 stated by the reviewer, we agree that these calculations were not described clearly enough in the original manuscript. The reviewer is correct that we converted FWHM to Ω_{Ar} for each of the 440 JCp-1 measurements, and then we took the average of these Ω_{Ar} values. This is clarified in the revised manuscript (page 8 lines 13-14 and page 10 lines 1-2). Additionally, we clarified that the JCp-1 analyses were conducted on the WITec instrument (page 8 line 13), as this may have been the source of confusion.

We also wish to point out that we have posted the .R scripts to repeat all the analyses in the manuscript and reproduce the figures at the following link:

<https://codeocean.com/2017/06/30/code-for-quot-coral-calcifying-fluid-aragonite-saturation-states-derived-from-raman-spectroscopy-quot/code>

The calculations that produce JCp-1 Ω_{Ar} of 12.3 ± 0.3 from the raw data are there.

Comment 4: Considering the large uncertainties of the source data (Omega from inorganic experiments), and the fact, that corals do not perform such experiments to grow their skeleton, it appears not realistic to claim the precise reconstruction of coral cf Omega +-1-2, as stated in the abstract. Despite this critique I am confident and look forward to see this published as a paper in BG.

Response 4: We agree with the reviewer that there are substantial uncertainties in Ω_{Ar} over the course of each abiogenic experiment, and this is discussed in detail on page 8. Nevertheless, the statistics of the calibration support our claim that Ω_{Ar} can be derived within 1-2 units with proper instrument configurations (page 1 lines 10-11 and Supplement). Furthermore, we have added more discussion of how we identified Ω_{Ar} as the primary driver of FWHM (section 4.1). We also acknowledge some uncertainty in how this can be applied to corals (page 13 lines 17-19, page 18 lines 1-6), although the available evidence does support the accuracy of our approach for deriving calcifying fluid Ω_{Ar} .

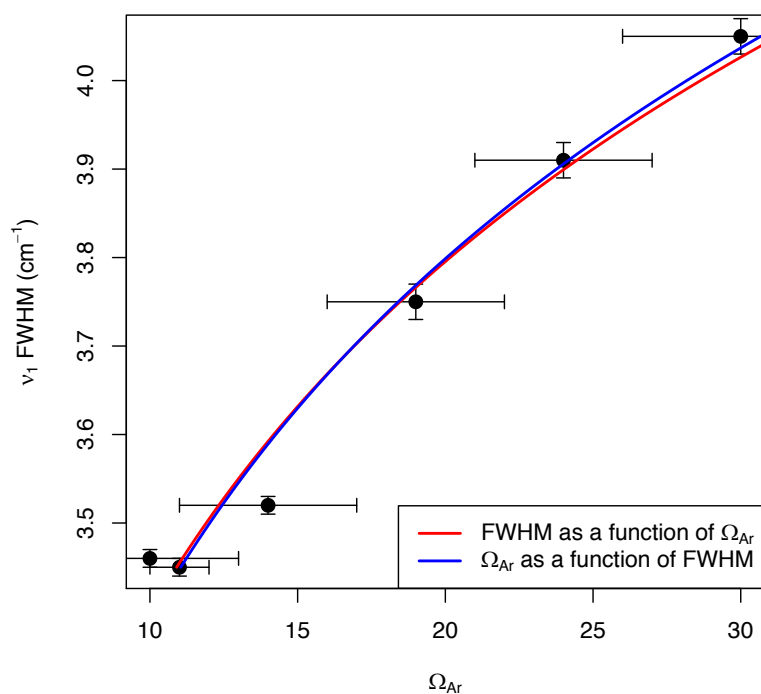


Figure R1-1 (also new Figure S6). Measured Raman FWHM plotted against fluid Ω_{Ar} for the abiogenic aragonites analysed with the WITec instrument. The red curve shows the calibration when FWHM is fit as a function of Ω_{Ar} , and the blue curve shows the calibration when Ω_{Ar} is fit as a function of FWHM.

Reviewer 2:

I have carefully read the manuscript by DeCarlo and others that describes the use of Raman spectroscopy to determine conditions at which (biogenic) calcium carbonate is precipitated. Overall, this manuscript contains an impressive amount of data, including results from inorganic experiments, culture experiment and field data. I have one serious concern about the (in)directness of the relation between ν_1 FWHM and the saturation state, that needs revising before this manuscript can be accepted for publication in Biogeosciences.

We thank the reviewer for carefully assessing our manuscript. The comments helped us to clarify how we conclude that Ω is the primary driver of ν_1 FWHM in aragonite. We added discussion of a subset of the abiogenic experiments with fluid $[\text{Ca}^{2+}]$ manipulation, which decoupled Ω from Mg/Ca and $[\text{CO}_3^{2-}]$. We are confident that our revisions satisfy the issues raised by the reviewer, and doing so has improved our manuscript.

Comment #1: The correlation between the Raman shift and the Ω may well be specific to the (inorganic) experiment and may not be general. As the authors explain in the introduction and methods, the actual shift in the spectrum is caused by other elements (impurities) or lattice distortions. In the well-constrained inorganic precipitation experiment (DeCarlo et al., 2015; Holcomb et al., 2016), the incorporation of impurities is apparently directly affected by the saturation state (since precipitation rate depends directly on omega and partitioning of elements depends directly on precipitation rate). Now, partitioning of elements (e.g. Mg, Sr) does not only rely on precipitation rate, but on a suite of other parameters, including temperature, seawater composition, photosynthetic activity and salinity. Not to mention species-specific differences in chemical composition of the aragonite (or calcite). This implies that changes in the ν_1 FWHM may change with Mg/Ca or Sr/Ca, but those are not always and only related to changes in seawater Ω in biogenic material. This does not defy the outcome of this study, but in my opinion does warrant a more careful discussion.

***Response #1:* The reviewer makes a good point that if trace element impurities (Mg/Ca and Sr/Ca) control the Raman ν_1 FWHM, then there could be differences between abiogenic aragonites and corals. However, our abiogenic results do not point to substantial effects of either Mg/Ca or Sr/Ca on ν_1 FWHM. In addition to our regression models, key data for decoupling Mg/Ca and Ω controls are two experiments conducted with elevated $[\text{Ca}^{2+}]$ concentrations (“f08” and “g13”), which reduced Mg/Ca but increased Ω . The ν_1 FWHM of these aragonites fall off the trend with Mg/Ca (revised Figure 3a and new Figure 5b), but are consistent with the Ω calibration (revised Figure 2 and new Figure 5a). We did not make the importance of these two experiments clear in the original manuscript, but we now highlight these results on page 12 lines 11-21. The Sr/Ca ratios of our abiogenic aragonites are primarily controlled by temperature (see Figure 3 in DeCarlo et al., 2015). If Sr/Ca influenced ν_1 FWHM, we should see a temperature effect with experiments conducted at 20 °C or 40 °C falling away from our calibration Ω -FWHM calibration, but this is not the case (Figure 2; page 12 lines 2-3), nor is there any correlation between Sr/Ca and ν_1 FWHM (Fig. R2-1 below). It is also important to recognize that the ranges of Mg/Ca and Sr/Ca (as well as other trace elements) in our abiogenic aragonites are comparable or larger than those found in most tropical scleractinians.**

The absence of clear effects of element/Ca ratios on ν_1 FWHM suggests that increases in ν_1 FWHM with Ω are probably driven primarily by disorder of CO_3 in the lattice. Although we do not yet have data to show exactly how this occurs on the molecular level, there may be a shift from highly crystalline aragonites forming at low Ω to relatively disordered or more amorphous-like aragonites forming at higher Ω . We included a thorough discussion (section 4.1) of the potential effects of Mg/Ca (and now Sr/Ca) because changes in FWHM of calcite and amorphous calcium carbonate are often attributed to Mg/Ca. Yet there is no evidence that this is the case for aragonite. We agree with the reviewer that translating any relationships from abiogenic experiments to corals must be done with caution, and we have acknowledged this in the text (page 13 lines 17-18, page 18 lines 1-6). However, our proposed FWHM- Ω calibration appears to be a general process of aragonite precipitating from seawater, independent of minor changes in trace element composition. We have no reason to think this is any less generalizable than using abiogenic element partitioning coefficients to interpret biogenic carbonates, and our testing with JcP-1 demonstrates the close agreement between Raman and trace element geochemistry. Nevertheless, we added to the Conclusion section that additional tests on corals will be useful. For example, checking for correlations between ν_1 FWHM and wavenumber is one way to test if element/Ca variability influences ν_1 FWHM of coral skeletons (page 18 lines 2-6).

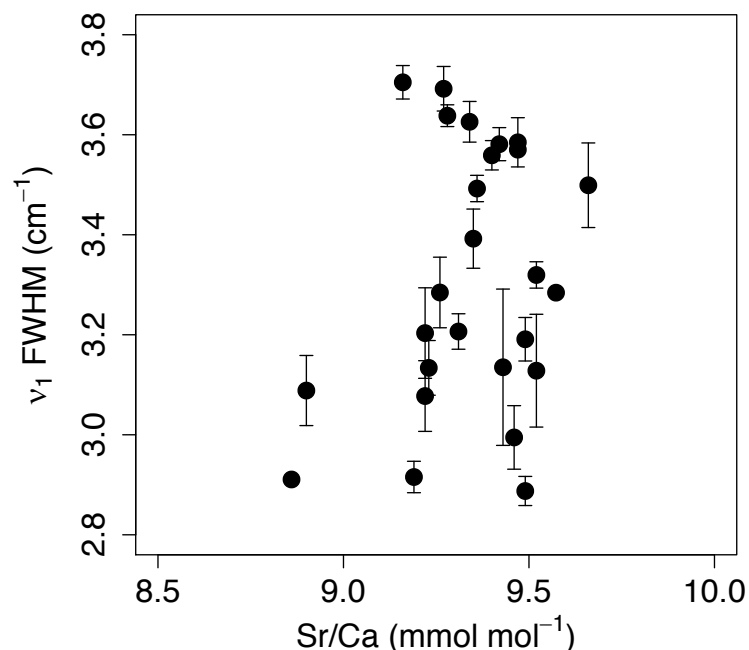


Fig. R2-1. Raman true ν_1 FWHM plotted as a function of measured aragonite Sr/Ca. There is no significant correlation ($p = 0.23$, $r^2 = 0.06$).

Comment #2: For example, the ‘apparent’ control of $[\text{CO}_3^{2-}]$ on ν_1 FWHM as shown in figure 3 may be just as real as that of Ω . Despite the outcome of the statistical modelling, both these two parameters seem equal explanations for the observed change in Raman shift, because both these parameters are (in a very similar way) responsible for the concentration of impurities in the aragonite. Therefore, the last sentence of the caption of figure 3 is misleading and needs to be changed.

Response #2: The reviewer is correct that variability in $[\text{CO}_3^{2-}]$ is difficult to isolate from Ω . While these two parameters were strongly correlated when comparing across all the experiments in our abiogenic study, the two experiments conducted at elevated $[\text{Ca}^{2+}]$ provide some independence (page 12 lines 11-21 and new Figure 5c). Like the discussion of Mg/Ca above, FWHM of these two experiments fall off the $[\text{CO}_3^{2-}]$ trend, but are consistent with our FWHM- Ω calibration (new Figure 5). Thus, while our results indicate that Ω is truly the controlling factor, we agree with the reviewer that $[\text{CO}_3^{2-}]$ should not be dismissed entirely, and we have revised our discussion to leave room for other possibilities (page 12 lines 18-21, page 13 lines 17-18, page 18 lines 1-6, and the Figure 3 caption is revised to "... patterns observed here *may be* artefacts...").

Comment #3: Using a similar reasoning, the coral's results do not necessarily reflect only (or even primarily) the saturation state of the fluid from which they calcify. The results are highly interesting, but the results from the inorganic precipitation experiments do not justify the interpretation spelled out by the authors. As an example: on page 10, lines 1-2 for example, the change in v_1 FWHM is claimed to solely reflect omega, which should be nuanced.

Response #3: We agree that some of the language in the original manuscript was overly strong, but nevertheless we stand by our interpretation that coral FWHM is likely controlled by Ω for the reasons listed above in responses #1-2. The reviewer is correct that other factors could theoretically have some effects (acknowledged on page 12 lines 18-21, page 13 lines 17-18, page 18 lines 1-6), but we have no evidence to support any factor other than Ω controlling FWHM of abiogenic aragonites or corals. We revised the manuscript to more clearly explain our reasoning that Ω is the controlling factor (section 4.1), while also stating that additional tests will be helpful to identify if there are subtle effects of other variables (added to page 12 lines 18-21, page 18 lines 2-6). Furthermore, we revised the description of the coral results (including the lines noted by the reviewer) to first state the changes in FWHM that were observed, and then we provide the changes in Ω assuming the abiogenic calibration applies to corals (page 10).

Comment #4: page 11, lines 6-8 When looking at the 6 experiments with varying temperature, only two experiments with different temperatures were conducted at the same Ω , according to figure 2. Is this enough to conclude that v_1 FWHM is not sensitive to precipitation rate? Figure 2b still shows a certain decreasing trend with increasing precipitation rate, especially when taking the average of the different experiments. Again, future work should be focused on a more decoupled experimental set-up, to also investigate the observed correlation with solid Mg/Ca and fluid $[\text{CO}_3^{2-}]$.

Response #4: The reviewer is correct that if taking the average of FWHM at each temperature, there would appear to be an inverse relationship to precipitation rate. However, since FWHM clearly increases with Ω (and precipitation rate) at a constant temperature, it would be very strange for precipitation rate to have both positive and negative effects on FWHM. That the data from all experiments fall along a single Ω -FWHM calibration (Figure 2) strongly supports that Ω is the primary driver, not precipitation rate (Figure 3b).

Comment #5: Page 12, lines 6-22: this may be a crucial paragraph since the authors describe here why the Mg/Ca of the calcifying fluid is not related to the ν_1 FWHM, but rather controls the incorporation of impurities and hence the average C-O bond length. However, this does not mean that Mg/Ca of the calcifying fluid could not have an equal effect on the Raman spectrum and hence, the measured ν_1 FWHM (e.g. in corals) may reflect either (or a combination) of fluid Mg/Ca and Ω .

***Response #5:* We agree with the reviewer that this discussion is pivotal to interpreting the coral data. Our new discussion of experiments conducted at elevated $[\text{Ca}^{2+}]$ adds to this argument. By changing the fluid Mg/Ca ratio, we achieved a factor ~ 2 change in aragonite Mg/Ca from ~ 5 to 2.5 mmol/mol but over a Ω range of only 10-12. This change in Mg/Ca covers most of the range of that found in shallow-water scleractinians (e.g., Gaetani et al., 2011 in *GCA*). However, there was no effect on FWHM. Thus, we can say that Ω was the primary driver of FWHM in the abiogenic aragonites (section 4.1 and response #1 above). Yet it is theoretically possible that Mg/Ca could cause subtle changes of FWHM in corals (page 13 lines 17-18, page 18 lines 1-6). This is a testable hypothesis because Mg/Ca will change FWHM and wavenumber in tandem (Bischoff et al., 1985; Perrin et al., 2016), whereas changes in FWHM driven by Ω are not associated with changes in ν_1 wavenumber. Thus, future studies on corals could check for positive correlations between ν_1 FWHM and wavenumber as a means of controlling for potential Mg/Ca effects (page 18 lines 2-6).**

Comment #6: Sections 4.3 and 4.4 may need to reflect another interpretation and rephrasing to avoid the suggestion that ν_1 FWHM directly and only reflects Ω .

***Response #6:* We revised the Results section to make it clear that FWHM was measured, and that we are interpreting these data in terms of the abiogenic Ω -FWHM calibration.**

In addition, there are some minor issues that I listed below that may help to further improve the manuscript.

Comment #7: page 2, line 17: saturation state is not the only thing that determines growth rates. Presence of inhibitors (Reddy et al., 2012. *J Cryst Growth* 352: 151-154) and the Ca:CO₃-stoichiometry (Nehrke et al., 2007. *Geochim Cosmochim Acta* 71: 2240-2249) also affect growth rates.

***Response #7:* We revised this statement to say that elevating Ω contributes to (rather than “drives”) aragonite nucleation and growth (page 2 line 18).**

Comment #8: page 2, lines 17-22: this is a bit of a stretch. First, the authors acknowledge that it is not certain (“if one exists”) to what extent the internal and external saturation states are related. Therefore, knowing the internal saturation state does not necessarily result in an accurate forecast of the fate of coral calcification (even when ignoring the response of coral biomineralization to other environmental changes). Please rephrase.

***Response #8:* We removed this sentence entirely as it was not necessary for our message and we agree that the sensitivity of calcification is likely more complicated.**