RC2

Response #1 We thank reviewer 2 for their opinion that our work represents a major advance in biomineralogy research. We go on to address their specific comments below.

The temperatures for A. islandica are worryingly warm, near the upper range of this clam's thermal tolerance (lines 179/180; Seawater was maintained at a pH of 7.93 ± 0.09, temperature of 18.2 ± 1 o C, and salinity of 35 psu for the aragonitic clam A. islandica in the control conditions (Downey-Wall et al., 2020). The authors need to provide this as a caveat to the results. In other words, are the results scalable for all temperature ranges?? The authors should consider the results of Liu et al. (2015) Environmental controls on the boron and strontium isotopic composition of aragonite shell material of cultured Arctica islandica, Biogeosciences, 12, 3351-3368, doi:10.5194/bg-12-3351-2015, whereby there seemed to be a potential influence of warmer temperatures on boron isotopes.

Response #2 Thank you for this point. *A islandica* and *C. virginica* specimens were maintained at different temperatures (9°C and 18°C, respectively) ; this will be edited in the revised manuscript.

Add the length of time for the experimental calibration for both species in line 180 at the end.
Response #3 We can add the length of time in the revision.

What are the ages and shell heights for the A. islandica shells? They grow very slowly, thus this is important to have these metrics in this study- (not just citing Downey-Wall et al. (2020))

"2.2 Calcification rate measurements Net calcification rate was calculated using the dry weight at the start and end of the experiment. Initial dry weight was measured at the start of exposure, on day 33 or 34, after the acclimation period (Downy-Wall et al., 2020). The buoyant weight was measured on either day 50 or 80 and the final dry weight was derived using a linear relationship between oyster dry weight and oyster buoyant weight (Ries et al., 2009)."

This may be suitable for juvenile mollusks but not for adults, especially A. islandica. What are the uncertainties in such measurements for large adult clams?

Why haven't the authors reported calcification rates for A. islandica. This is a central variable that needs to be considered (like Fig. 2a for oysters).

Response #4 We will clarify that calcification measurements were only conducted on *C. virginica* specimen, not *A. islandica*. Our calcification measurements were taken to understand how

calcification was affected by ocean acidification treatments, which *A. islandica* were not exposed to.
Shell sizes can be added to the revision of the materials and methods section and a better explanation can be provided. We were interested in the geochemistry of the shell and EPF of *A. islandica* specimens under ambient CO_2 conditions. The *A. islandica* specimen grew under ambient CO_2 conditions as they were collected in their natural environment. Our need to maintain specimens under laboratory conditions was specifically to examine the EPF fluid and sample while in controlled conditions.
The longer term experiment for *C. virginica* was needed to have the faster growing *C. virginica* which would have laid down new growth under CO_2 treatments.

How are the authors confident that they sampled ONLY calcium carbonate reflecting the experiment? Did they stain the shells with calcein? Did they measure linear growth? This is most relevant for A. islandica because of relatively slow growth rates (i.e., see Liu et al., (2023) Resistant calcification responses of Arctica islandica clams under ocean acidification conditions, Journal of Experimental Marine Biology and Ecology, https://doi.org/10.1016/j.jembe.2022.151855.)

Response #5: We sampled a thin layer across the inner surface at the base of the shell, avoiding any parts such as repaired shell laid down to cover bore holes, as we thought the chemistry of those regions might be different. We don't have info such as a calcein stain to show where the new growth is, so we sampled shell material that was in close contact with the EPF and was recently laid down. But we acknowledge that can be a potential bias.

Shell sampling – the organic matrix in shells contain about a magnitude more boron than in the shell, and this likely has a very different isotopic composition (value). Are the authors confident all organics were removed?

Response #6 Yes, before isotopic analysis carbonate samples went through two oxidative cleaning steps to remove organics from the carbonate material. The oxidative agent consists of H2O2 which is similar to other studies (Pre-treatment effects on coral skeletal δ13C and δ18O - ScienceDirect). This cleaning was performed on various marine organisms and led to consistent data within species (corals, coralline algae, oyster, Liu et al. 2020). We can add a sentence before line 233 to explain this in more detail rather than just stating " 2.5-3.0 mg of oxidatively cleaned shell powders were dissolved in 1N HCl."

Why are the authors explain how they sampled the oyster shells but not the clam shells? The methods should have a parallel structure.

Response #7 The sampling was performed in the same way for both bivalve species, this can be added into our methods section for clarity.

Very interesting result in Figure 3- showing different chemical composition of EPF compared to ambient seawater. Important finding that lots of folks have been suggesting but without the EPF evidence. And when you go to the shells even less Mg than seawater, and EPF. Thus the mollusks must be regulating calcifying fluids.

I really think the authors are missing an opportunity by not exploring changes in the shell geochemistry from both species here with growth rates, shell height, age, etc. The applicability/scalability of the study is far less without the inclusion of such metrics. Why not include these data?

Response #8 Some of those data were not collected for both species. We have calcification data for *C. virginica* however calcification was not recorded for *A. islandica*. With the data we do have, we can re-explore those metrics and add them to the revised manuscript.

Ok- now some praise for the authors:

This is an important study with important implications. We learned that oysters (C. virginica) and clams (A. islandica) incorporate some elements and boron isotopes differently. The boron isotopic composition of the EPF for both species is different than seawater. The breakthrough of being able to sample the EPF chemistry/pH is a major advance in biomineralogy. Thus, a mechanistic model for biomineralization can be advance. Also, the mollusks evaluated here are not simple pH meters, and the shell d11B value is a mixture of the seawater d11B value and physiology. These results are consistent with in prep work that I am aware of now. Despite some issues with the description of the experiment and other concerns noted above, this is a major advancement.

References cited in response:

1. Grottoli, A. G., Rodrigues, L. J., Matthews, K. A., Palardy, J. E., & Gibb, O. T. (2005). Pre-treatment effects on coral skeletal δ13C and δ18O. Chemical Geology, 221(3-4), 225-242.
2. Liu, Y. W., Sutton, J. N., Ries, J. B., & Eagle, R. A. (2020). Regulation of calcification site pH is a polyphyletic but not always governing response to ocean acidification. Science advances, 6(5).