

The manuscript is much improved from its previous version, and I appreciate the author's efforts to clarify several points. I will highlight a few key things and a few outstanding minor issues, but if the authors are prepared to stand as firmly on their conclusions, then far be it from me to prevent them from doing so. I don't believe the disagreement and differences in interpretation prevent the paper from being published. I do however feel strongly about an issue here, in terms of their mis-interpretation or incorrect presentation of aspects of the literature, I will highlight a few examples, and note more at the end of my review. This is perhaps the most damaging component of the paper that needs to be corrected prior to publication.

As the authors clearly acknowledge, there is no way to experimentally disentangle saturation state and the ratio of bicarbonate to protons, they are bound by the second dissociation constant of the carbonic acid-base system. We are therefore left to argue points based on interpretation of the published literature and current understanding of calcification mechanisms in bivalves (which vary considerably from other species such as corals and coccolithophores). However, the entire argument is based on the idea that total DIC becomes limiting at values below 1000 umol, and above that the build-up of excess protons and the inability to remove them from the calcification front/cells further inhibits calcification. Since the authors note that there are very few to no habitats in which total DIC is below 1000 umol, then if we consider values closer within the range of what may be found in natural environments, we are left, following their argument, that it is pH that actually drives the response. It is harder to see this interpretation of the data with the data available through these experiments.

Unfortunately the authors misinterpreted my criticism of the lack of treatments and inferential power. I did not state that the lack of treatments did not allow the authors to answer any specific research question, rather I cautioned the authors in the context that throughout their manuscript they are arguing for/against 4 different variables, and a ratio of two of those, with only 4 treatments. Ideally you would want at least one more treatment than the number of explanatory variables in the experiment. The system is woefully under-constrained, and as I noted above, this doesn't prevent publication of the data, but rather I would have hoped the authors take a more cautious approach in the interpretation of inferences drawn from their experiments.

Returning to the issue of whether saturation state or the ratio of bicarbonate to proton concentration matters, I would direct the authors to more carefully look at the results in our 2014 publication, and consider developing of the early larval stage, in addition to calcification/growth. For the oyster larvae, we see normally developed larvae in conditions with bicarbonate concentrations below 1000, and significant abnormal above this. (and in the mussel experiments we see plenty of examples of abnormal development above 1000 when pH is not terrible). In addition, we found a wide range of sizes (calcification) within bicarbonate ranges associated with saturation state, unfortunately, as noted above, once bicarbonate concentration is held nearly constant, then pH and saturation state will co-vary. So what I am after is a fundamental mechanism of proof that calcification (never mind development for now), is in fact diffusion limited, that is the fundamental argument, if I understand properly, that calcification is either reactant limited (by DIC), or inhibited by build-up of protons at the calcification site/cell. The ratio, as I understand it is also important when DIC is not limited because bicarbonate somehow helps with proton removal. While all of this is plausible, it is simply striking that the inflection point of this relationship is so close to the saturation horizon. The second part of this is whether the calcification fluids are in fact isolated as an entirely enclosed body compartment.

On to the ability to isolate the calcification fluids from the external environment, as if we assume diffusion itself is allowing products to escape the actual calcification surface rapidly, then it must be a build up in the fluids, as the authors do note. While the authors note little is in fact well documented in early larvae, there is considerable work on this issue in adult bivalves. While mussels do in fact seem to seal the inner and outer EPF quite well (as has been the primary bivalve taxon the authors have worked

on), anatomical differences across taxa prevent all bivalves from doing this to the same degree. There are several papers in the older literature on this, and we have summarized this in follow up work to be published in less than 2 weeks. Lines of evidence come from bacteriological studies, mass balance calculations, and anatomical differences across taxa. I will forward a copy of the manuscript to the lead author. And it should also be noted, some recent work on corals, which are believed to even more skillfully protect their calcified structures with their tissue, appears to let tracers, which cannot pass through membranes, to the sites of calcification.

So while we disagree on the importance of saturation state versus the bicarbonate/proton ratio, there are other lines of evidence that also undermine the staunch position the authors have taken. I would not argue that there are no cases where say DIC concentrations may be important, not acid-base regulation of internal compartments, and we noted such in our previous work. I also believe they have misinterpreted our findings and argument for the role of saturation state, or argument is not about substrate availability, as we have clearly acknowledged that bivalves use all forms of DIC to make shell in our previous studies. Rather, the kinetic rate law for calcification puts a constraint on the organism, making it harder (more energetically expensive) to create shell material under decreasing saturation state. This is a subtle, but important difference between previous papers arguing for the role of carbonate ion concentration in calcification (due to its role as a reactant) versus a kinetic constraint on the calcification at higher reaction rates.

Again, if they choose to hold-fast, then I applaud their certitude about processes that we still fundamentally know very little about. Our arguments have been directed towards only bivalve larvae during the earliest shell building stages, and we have shown that the response of development also responds to calcification, most of the larvae in our studies do in fact create shell, but low omega prevents them from making a calcified structure properly.

Minor comments-

To be clear, while pH and carbonate ion concentration are correlated, it is not correct to say pH affects carbonate ion concentration. This is a fundamental misunderstanding of the marine carbonate system. The carbonic acid-base system is the primary (but not only) buffer in sea water, and it is the speciation of the carbonate system that largely controls pH. The way that pH may impact carbonate ion concentration, is with a large disturbance of a different acid-base system in seawater. It would be better if the authors do not perpetuate this mis-understanding in the literature by using precise language.

Regarding the isotopes and shell incorporation, it is important to note that during PDI shell formation, mass-specific metabolic rates rise rapidly during and decline sharply after, and while once would anticipate higher metabolic C incorporation during this stage, it is not seen. While calcification rates may in fact be relatively higher in relation to respiration, since we lack direct estimates of both in a single organism simultaneously, I would caution against entirely dismissing the entire argument. This is the argument presented in Figure 5A from previous data in Sprung 1984, however the units are unclear at this point, are these standardized to C? And there are many other papers that include similar data that would perhaps not show as clear a relationship, so is it valid to show only these data, and exclude other measurements of respiration rate and shell calcification in similar species?

Regarding shell thickness, the authors are correct in that smaller organisms generally have thinner shells, but shell thickness varies across the length of the shell. And the question then is whether organisms will extend shell when they cannot maintain current shell due to internal dissolution. The lack of a measured response doesn't mean it cannot exist, and others have clearly shown differences in adult bivalve shell length to thickness within species. We don't know if this is true in larvae, but again, it would seem prudent to at least acknowledge the possibility, essentially an assumption of the argument laid out.

Comment- “Page 1554 Our study deals with bivalve calcification”, but the arguments for the bicarbonate/proton ratio are detailed in other studies, with the current argument relying very heavily on those.

Comment- “We do not see a realistic scenario in which...” most estuaries are rarely in equilibrium with the atmosphere. In fact many estuaries with low alkalinity can have pH values above 9, due to photosynthesis related to algal blooms.

Comment- Line 23 in the revised ms... It is good that this was included, but the interpretation of the Pan et al. study is incorrect here. The authors noted that acidification reduced the energy available for protein synthesis, however, Pan et al. show that under acidification a large increase in energy allocation towards protein synthesis occurs, with little change to ion pumping. This half aligns with the previous work by the authors about the lack of internal regulation of pH, but mis-represents the findings of the Pan et al. study.

Figure 5 caption should include reference to the data used to generate Figure 5A. It is also unclear what the units are here, are they in pmol of C or simply pmol of oxygen and CaCO₃. This is also true in the written sections, it would help the reader if the authors were explicit. I’m wondering why they also excluded the data from Widdows 1991 to include in their respiration/calcification comparison? Nor do they state at which cell density the shell growth data from Sprung are used in the figure.

In the paragraph starting “Earlier studies...” the authors note our hypothesis regarding the ability to isolate the shell as part of the reason for the sensitivity to external carbonate chemistry, and then note that the calcification responses are similar between early larvae and juveniles. This again oversimplifies our study and interpretation, while we noted the differences in shell length of normally developed larvae, we also noted the failure of normal development (but still building of shell), due to low omega. We also included the argument for this sensitivity is the limited energy available until completion of the first shell, and the rapid rate of shell formation at this stage. While I agree that there is a response in juveniles, we have never argued that the heightened sensitivity at the early larvae stage precludes responses later in the larval or juvenile stage. And with the limited number of treatments, the authors are incapable of fitting a functional response of a meaningful fit. Rather, all their data state is juveniles are also sensitive at very poor chemistry conditions, whether there is a different threshold is something their data cannot address (but is essentially what they are stating).

At the end of this paragraph, the authors go on to note that passive diffusion of CO₂ to the calcification site would be responsible for the patterns we noted in our 2013 paper. It is important to note, mass specific metabolic rates increase rapidly during PDI shell formation (See Widdows 1991), the period when we see the lowest incorporation of metabolic CO₂ into the shell. As the sentences are currently written, it seems to imply the opposite. While they are correct that the balance of respiration to calcified C will to some degree determine this (as highlighted by Lorraine et al.), additional lines of evidence suggest the earliest shell is in greater contact with the external environment (as noted above).

In all, I don’t believe my concerns should prevent publication of the manuscript. I would however like to see a more careful treatment of the literature. In a few cases, as noted above, the authors mistakenly represent findings from a study, or over-simplify the conclusions. Other examples include noting Moran and Monahan 2004 and effects settlement success, this was never measured in this study, ultimately the authors found respiration but not growth can be supported presumably by DOM, and once food is added, growth increases. But the authors never measured settlement (but a significant body of literature has shown energy as a strong predictor of successful metamorphosis). In the context of calling into question whether environmental stress will limit the larval energy budget and manifest in later life impacts, there are dozens of papers, some of which are cited in the papers cited here, that show low energy stores affects

the ability of larvae to metamorphose (settlement is typically used to refer to the act of selecting a habitat). And finally, I believe the authors also mis-interpreted the findings of Hettinger et al. 2013, and appear to cite Riisgard 1980 for studies on *Ostrea lurida*, which is clearly not the case (and it is not included in the reference list). With regards to Hettinger et al. 2013, the authors did find a food effect, and CO₂ effect, and food did not entirely ameliorate the CO₂ effect, and the sizes did not in fact level out at the end of the experiment. So I am unclear how what they wrote relates to the published work, I'm unclear if maybe this is a typo. The authors should also note here they exclude other previous works that have shown significant OA carry-over effects (Hettinger et al. 2012, Barton et al. 2012), as well as significant reviews on larval carry-over effects by Pechinik).

But the manuscript is vastly improved from its initial version, and it should be suitable for publication in Biogeosciences with some careful revisions, particularly properly citing literature, as thus providing a more nuanced and complete manuscript, that clearly acknowledges the limitations and assumptions behind their arguments.