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Interactive comment on "Impact of seawater carbonate chemistry on the calcification of marine bivalves" *by* J. Thomsen et al.

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Review of Thomsen et al. "Impact of seawater carbonate chemistry on the calcification of marine bivalves"

Thomsen et al. provide results from a study geared towards understanding which component of the carbonate system is most important to 2 species of larval bivalves, and one juvenile species. They ran experiments by stripping alkalinity with a strong acid to lower alkalinity, then bubbling with CO2 to equilibrate conditions. Following the experimental work, carried on only one species during the initial larval stage and during the juvenile stage, the authors compile data from other studies on 2 larval bivalves to show the relationship of growth to carbonate ion concentration. Ultimately, there is value in this work, however, I see several significant deficiencies that require a substantial revi-

sion before it should be considered for publication. This is unfortunate in my opinion, as these authors have typically produced well thought out and insightful work in the past, but this manuscript is lacking in several key areas, and would benefit from more effort to better clarify the arguments in the discussion. I had communicated with one of the co-authors prior to this review, as the work presented here (and several of the arguments) are remarkably similar to Waldbusser et al. 2014, which was published online at Nature Climate Change (http://dx.doi.org/10.1038/nclimate2479) two days prior to the submission of this manuscript. The authors were aware of this paper, and it is unfortunate they did not regroup and account for this work in the context of the manuscript, as I believe it would have benefited this manuscript tremendously. Along these same lines, the authors fail to properly attribute several key ideas in a previous manuscript (Waldbusser et al. 2013, GRL), that would support several of their statements, even though they cite the paper within their manuscript. To be clear, that paper, explicitly notes a kinetic hypothesis for the sensitivity of early bivalves to ocean acidification. I have noted below in the detailed section, several other mis-citations of other papers, and there are some other key citations completely lacking, such as Salisbury et al. 2008, Hunt et al. and Cai et al. all of which address the complexities of alkalinity in estuaries.

One of the key weaknesses of this paper is the authors have several variables of interest, and very few degrees of freedom to draw inferences from. With only four treatments per experiments (and one with three), the authors really have at best a two way ANOVA with two levels of each factor (PCO2 and carbonate ion concentration). What is unclear, is if the author's hypothesis was bicarbonate ion concentration would be the primary factor of interest, why not design the experiments to test this effect? As such, the alkalinity concentrations, that are argued for being so important, are only replicated at one level in two of the three experiments, and not at all in the third experiment. In the two experiments where alkalinity is replicated for a given carbonate ion level (but not PCO2), there is wide deviation in the response. Had the authors had bicarbonate/alkalinity concentrations across the range of carbonate ion concentration, perhaps

their inferential power would be greater, and they could support their conclusions better, but currently these seem tenuous at best.

The authors go on to build an argument based on the bicarbonate/proton ratio (which is correlated with carbonate ion) as being the fundamental explanation for the results here. While there is some evidence for this in previous studies, as they noted, those have been primarily on different species with very different modes of calcification. While they fail to acknowledge the true mechanism for the kinetic hypothesis previously described in Waldbusser et al. (2013), as being dependent on saturation state (due to the kinetic rate equation). It would behoove the authors to acknowledge both possibilities, given there is not yet a clear way to full separate these two possible explanations for the results that appear to be related to saturation state.

There are several components of the methods that are missing. How many organisms were sampled? How many replicate experimental units were included in each experiment? There is lack of definition of the criteria utilized to collect juveniles in the field (e.g. were the authors looking for individuals above a certain size?), although later on they referred to them as 2-years old individuals. This aspect needs to be more clear, as it is expected that the sensitivity to ocean acidification stress during the juvenile stage might vary depending on the time past metamorphosis.

The timing and dates of experiments are also important, to determine the general condition of broodstock. Along the same lines, it is key to state the duration of each incubation. For experiment 1 there is conflicting information: In page 1548 line 5 and 6, the authors state that the incubation lasted for three weeks. However, in the same paragraph line 15 they say it was terminated after 15 days.

For an experiment that measures the responses to variable carbonate chemistry, the details of the chemistry sampling are sparse. When were the discrete samples taken? Before and/or after the incubations? How many replicates? Were the samples analyzed immediately after collection or preserved and analyzed afterwards?

Finally, there is virtually no detail on the methods for the meta-analysis presented here, until the results, where some details are laid out. However, it is unclear how the authors corrected for differences in temperature across studies? It is clear that the authors used percent changes from controls, but never try to correct or account for different experimental temperatures and salinities. Note published growth models for C. gigas larvae show very strong temperature and salinity impacts on development and growth, and the studies in the meta-analysis use a broad range of temperature conditions, nearly 10C. There is some potentially important value to this approach, but more details are needed on how the analyses were conducted.

Along these same lines, the authors missed several other papers on C. gigas larvae, or fail to explain why they were excluded. I would advocate to expand this portion of the work to include other papers and species, as this would only help clarify the points raised, and bring up some important aspects of other covariates that have often been ignored in previous larval work (that are very important). There are a few studies that have looked at acidification impacts on the same species under different salinities, which would provide more appropriate comparisons. Finally, regarding the methods for the meta-analysis, I recognize the challenges of comparing across different studies, but forcing the results into a percentage, puts an upper limit on the response, begging for a logistic regression (as this is a 0-1 response now). I worry that by bounding the upper level, numerically this limits the potential for greater responses. The data in Waldbusser et al. 2014 show a power function, not asymptote at much higher carbonate ion concentrations, in the same experiments with the same organisms. How might one resolve these differences?

Another issue that the authors should at a minimum acknowledge is the use of a relationship between respiration to shell growth to estimate a calcification rate. Several papers cited within the manuscript, and others raise some concerns with respect to assuming that respiration rate can be used to predict calcification. The assumptions (and limitations) of such an approach need to be clearly articulated. If there is an increased

energetic cost for calcification under acidification, it would seem that this relationship would change? Perhaps I missed something in the manuscript, but others have shown that shell extension doesn't always mean increased shell thickness, another assumption required for this approach.

Two arguments that the authors make through the discussion seem to become tangled, and ultimately fall apart. First is the energetic cost of calcification under higher CO2, and second is the importance of the bicarbonate/proton ratio. The pumping of protons is not energetically expensive relative to protein anabolism for shell formation, this was cited by the authors (Palmer 1993), previously argued by the authors (Thomsen et al. 2013), and was clearly articulated by Waldbusser et al. 2013 for developing bivalve embryos. The relative energy then needed to incorporate more metabolic carbon versus DIC seems trivial to the process. I would argue that we however do not have a solid handle on the true energetic costs of shell formation, and if the kinetic hypothesis (noted previously) is correct, we need to develop new models to account for the acceleration of precipitation by fauna (as noted in Waldbusser et al. 2014). The authors however, after noting the cost seems to lie in protein formation, argue that bicarbonate/proton ratio is really important, then to ultimately argue that carbonate ion concentration appears to be the key (but this is correlated to bicarbonate/proton). We lack the ability to experimentally separate these two possibilities at the moment. It would greatly increase the importance of this work if the authors would more clearly articulate both, and the evidence for both, rather than ignoring evidence that is in some of the references cited in the manuscript.

I would strongly encourage to examine the data from Waldbusser et al. 2014, in the supplemental material, as these studies show that increasing DIC within a saturation state (or carbonate ion concentration) does not have any significant effect on development or growth. This experimental data is the only data I currently know of that explicitly tests the total DIC effect. Given the correlation between bicarbonate/proton and saturation state, we cannot explicitly separate those effects.

What I cannot seem to understand is why a given absolute concentration of carbonate ions would be so significant, and how that would be supportive of the bicarbonate/proton ratio being the root mechanism. It seems more likely that saturation state may be important here, given that concentration is likely near the saturation horizon, but adequate data are lacking to evaluate in the current study. Perhaps I missed something, but given the conflation of these concepts in the paper, I would strongly encourage the authors to spend more time working out the logical arguments here.

I should also note that the only paper I am aware of that tested the potential effect of carbonate ion versus saturation state lacks appropriate controls for the Ca addition used (Gazeau et al. 2011). The work by Waldbusser et al. 2014, elevated aragonite saturation state from approximately 2-4 by a similar Ca addition as in the Gazeau study and found worse growth and development than all carbonate chemistry conditions. Ca is key in several cellular processes, so it is not surprising that rapidly changing the background Ca levels resulted in very poor development and growth. This doesn't mean the conclusion is entirely wrong, it simply means we lack the experimental capacity to determine saturation state versus carbonate ion by Ca addition. So while the authors focus solely on carbonate ion concentration, they clearly note that the entire DIC system is incorporated into shell carbonates, and there is equal support for a saturation state sensitivity (and this needs to be acknowledged).

Ultimately, I hope the authors can more fully reason through the arguments made in the current paper, and provide a more comprehensive vision on the work and challenges ahead. I fully agree with the final sentence of the paper, that there is much work still to be done to understand the mechanisms and dynamics of larval calcification and its sensitivity to ocean acidification. However, as the manuscript is currently presented, it suffers from many deficiencies that need to be attended to before it should be published.

Detailed comments-

The introduction seems to lack focus and structure, and departs from previous arguments made by these authors, while lacking many references that would support their points.

Page 1545 Line 8- “It has been hypothesized. . .” by whom? Most of the good physiological work, including by these authors, have focused exclusively on the ability to regulate internal acid-base chemistry. If the argument is later made that calcification occurs under controlled conditions, without exposure to ambient seawater, how then does ambient water carbonate ion concentration affect calcification? Line 10- “Strong undersaturation (W < 1)” anything less than 1 is undersaturated, above is super saturated, strong undersaturation is a qualifier that is in-definable. Line 15- include in this list Waldbusser et al. 2011 (eastern oyster responses to CO2 at different salinities, and Dicksen et al. 2012). Line 25- See Waldbusser et al. 2013, for a very clear and well articulated hypothesis of why saturation state may be so important to at least bivalve larvae: rapid calcification, limited energy, and greater exposure to ambient water during PDI formation. Line 27- “Its [CO32-] availability is highly variable due to the strong dependency on seawater pH and concentrations drastically decline at pH values below 8.5” . “Strong dependency” implies that pH is a master variable that drives the carbonate system speciation. This is false. pH is a consequence of the speciation of the DIC system.

Page 1546 Line 2- Cite who has suggested direct CO3 impacts on calcification? Line 6- There are several studies that also point to the lack of precursors in calcification, this is a not yet fully resolved issue for mollusks, and see Mount et al., and others. Line 19- “Gobler”, cite also Barton et al. 2012, and reviews by Parker and Gazeau both in 2013 Line 24- open ocean salinity is almost never above 35 psu Line 29- Cite Waldbusser and Salisbury 2014, directly reviews to salinity variability effects on carbonate chemistry of coastal environments, and it is unclear if any of the citations actually note that variability in those systems will increase.

Page 1547 Line 1- Salisbury et al. 2008 is a far better reference here. Line 4- al-

most all estuaries have residual alkalinity, that is the primary source of alkalinity to the oceans over geologic timescales. However some estuaries may have double oceanic alkalinity due to carbonate lithology in the watershed, while others high due to presence of organic acids, and others still much lower. This should either be broadened to encompass the full nature of alkalinity in estuaries, or removed entirely. Line 9- See Waldbusser et al. 2011, direct measures of juvenile oyster calcification under CO2 treatments applied to waters of different salinities. Line 12- but juveniles make of 1/3 of the experimental studies. . . Line 13- what does “strong” mean here? Line 13- “specific carbonate system parameters” is not explicit enough. This is the ending paragraph of the introduction, and therefore a more detailed explanation of what the experimental treatments were is warranted. Line 20- What were the criteria to select juvenile specimens? Threshold size? Line 20- Please include the complete scientific name of the species the first time you refer to it. In this case, Mytilus edulis.

Page 1548 Please include dates of experiments Please include numbers of individuals in experimental containers Please include number of replicates of experimental containers Line 5- Replace “units filled with 0.2 μm seawater” with “units filled with 0.2 μm filtered seawater”. Line 6- State the species or replace Rhodomonas with Rhodomonas sp. or Rhodomanas spp. Line 15- Contradictory information about the duration of the experiment. Line 5 says “The experiment lasted for three weeks”, while line 15 says “The experiment was terminated [. . .] after 15 days.” Please correct the confusion. Line 23- Provide reference for the overestimation of calcification rates less than 10%.

Page 1549 Please include dates of experiment Please include number of individuals in experimental containers and number of replicate experimental containers. Line 2- How many adult males and adult females were used for the spawning? Were there any measures taken to avoid polyspermy? This can be a very important issue when spawning bivalves in aquaculture or experimental systems. Line 8- Besides the number of cell divisions, the authors should note how many hours post-fertilization the embryos were transferred into experimental units. The hours post-fertilization is an indicator of

how fast the embryos were developing. Line 10- “The experiment was terminated after the D-veliger stage was reached in all treatments (day 4)”. The authors should clarify if they allowed the larvae to develop until day 4 to make sure that they have reached stage prodissoconch II or if actually they observed that not all larvae had reached D-veliger stage until 4 days post-fertilization. If the latter case Line 10- feeding at 2 days is usually seen under typical development, is it possible that not feeding until 4 days, when energy is at a low would bias results. This possibility should be addressed in the discussion. Line 22- When were the carbonate chemistry samples taken? How many replicates? Were the samples analyzed right away or preserved and analyzed later? If preserved, how were they preserved?

Page 1550 Line 4- Using published data on similar but not the same species for calculating calcification rate, based on size falls into some pitfalls. See work by Gaylord et al. that shows differences in shell thickness, and previous work by the authors also has demonstrated evidence of dissolution on adult shells, possibly biasing these estimates. It would be better to present data in the primary units of measurement and note these are a proxy for calcification. . .

Furthermore, this becomes even more tenuous when calcification rates are estimated by respiration rates in other experiments (there are a number of studies on bivalve larvae respiration that show some similarity, but a fair bit of variance under similar conditions). At a minimum, the authors should clearly document the assumptions of this approach, which includes assuming that excreted growth will follow respiration perfectly. Others have documented clear disconnects between shell and tissue growth, and if the authors are arguing that pH regulation is an important part of the physiological response, then logically, one would assume increased the scope for growth has to somehow vary perfectly with respiration and shell growth. It is also unclear how or whether the authors adjusted their respiration rates for possible temperature effects, this is an obviously important factor to make this link. I would recommend better defending the rationale for this, or dropping this from the study. Further, see work by

Vargas et al. on feeding responses of bivalves to OA, and the assumption that consumption remains constant under different CO2 levels is also tenuous.

Line 21- the meta-analysis is one of the potentially very strong points of this study, and I would argue that expanding this to include other life stages and species would vastly strengthen the usefulness of this study to the community. There are several papers on related species, at different life stages, that would help fill in gaps here. Even still, the authors are missing several other papers on C. gigas larvae for this study, including papers by Timmins-Shifman, Barros et al. I am currently lacking the supplemental material, but the authors need more detail here on how the corrected data. for example, many of these experiments have been run at far from optimal culturing conditions for c. gigas, how are those corrected? The controls in those experiments do not have a temperature control. So how is this fundamental physiological process, that others have shown to be dependent on temperature, corrected across these studies? The compiled data are incredibly cohesive, so it is hard to imagine that the data were not somehow corrected by temperature, given the temperature range of these experiments is nearly 10C.

Results- The experimental design is lacking in a few measures for interpretation. As noted above. Also, it would help the reader follow the work if the actual species name is used when describing results from each experiment.

Page 1552 Line 1- Is there a citation for this? See Timmins- Schiffman, but other than that, I don’t know of any studies that support this, and this is a statement that needs to be supported by a citation. Line 12- The authors seem to be avoiding saturation state as a variable, and do not even address this in the paper. Line 15- check periods and comma usage here for the bicarbonate ion concentrations. Line 15- Also, some of the conditions noted here I cannot find in the table of experimental conditions. For example, pH 8.90 is noted here as a pH of one of the treatments. I cannot tell if this is a typo, or some other issue. Is this because different pH scales are reported in the paper? Line 18- Again, the carbonate ion concentration is not a consequence of the

high pH, but a cause.

Line 1553 Line 1- why not plot these as saturation state? Without more information on the meta-analysis, it is hard to interpret these results. By forcing these results into a percentage it creates a logistic regression approach. The statistical approach to determining the threshold seems a bit unusual, given the results in some of the actual studies show increased calcification at higher carbonate ion concentrations. I would like to see more justification in the methods for this approach (not listing the methods in the results), and a clear statement about how effective this approach is for data bound between 0-1.

Line 20- This comparison seems odd, since the calcification rates are derived from respiration rates, and thus if there wasn't agreement, something would be very off. As I noted above, the relationship between growth and respiration doesn't have to follow, and it is surprising the authors try to use it here, given their very nice work previously discussing the changes in scope for growth under different CO2 conditions, and the changes in respiration rate due to excess CO2. It is unclear to me how one is to fully interpret these results.

Following here, the authors note similarities in individual based respiration rates. What becomes confusing is the various jumps between relative rates, individual based rates, and mass specific rates. I would strongly urge the authors to clearly separate these, and discuss why these are different, and what that actually means.

Page 1554- This is not the first time anyone has noted bicarbonate is the primary substrate used for calcification. Papers date back to the mid-1990's highlighting this, and has been the subject of many studies on corals and in other species. Additionally, some of the papers cited in the discussion on isotopes have made this case previously. Line 3- Sure calcification is affected here, but there are plenty of other studies that show other physiological processes are affected.

Line 10- This was the point made by Waldbusser et al. 2013. Line 17- Also Waldbusser

et al. 2013 Line 20- This is an incorrect citation for this paper, there is absolutely no discussion on space limitation in Waldbusser et al. 2013. It also misses the point of the paper, by mis-citing the findings to better support the story being woven here. Line 21- The authors argue that CT is the primary substrate, but then come around to carbonate ion concentration, but given carbonate ion is such a small portion of the CT why is this important? And again, the lack of increase in calcification above 80 uMol is likely an artifact of the approach used to standardize the calcification data on a scale of 100%. In the papers used for the analysis there is an increase, it just slows. Additionally, the experiments of Waldbusser et al. 2014, show a true power-function response to saturation state (carbonate ion concentration). Line 25- Waldbusser et al. 2011, Dickerson et al. 2012, Salisbury et al. 2008, The following interpretation needs to be re-thought in particular, because the limited treatment matrix, and the lack of degrees of freedom to make this interpretation. Without better orthogonality among variables, this is a very tenuous conclusion.

It is also important here to be clear about total alkalinity versus total DIC. Although those are closely related in most marine and estuarine waters, there is the potential for non-carbonate alkalinity in some systems (see Cai et al. and Hunt et al.). One could envision a similar experiment where a strong base is used to increase alkalinity, without total DIC.

Page 1555 Line 3- This again, is well worn territory, not a new concept that total DIC (or bicarbonate ion) is important for calcification. See Ries paper on physiochemical model of calcification. Some of the isotope papers cited here have well documented that, and include many references that further state that. Also, the comparison with other taxa, is a bit tenuous, given the very different modes of calcification. Line 9- the primary reference for this is Mount et al. Line 14- the importance of bicarbonate does not hold for other bivalve larvae in experiments done across a wider range of conditions. In Waldbusser et al. 2014, at similarly low saturation states, a range of increasing CT from roughly 800 to 2000, little difference is seen in growth or development. Line 15-

The tenuous argument being built here starts to crumble here, as the authors note that acidification increases CT, but lowers carbonate ion (or saturation state), and it is the decrease in carbonate ion concentration (or saturation state) that the organisms respond to. I encourage the authors to look at the experimental treatment matrix in Waldbusser et al. 2014, and they will see that that this argument doesn't hold entirely. In that study, there was a minor effect of total DIC on larvae, but it was a fraction of the effect of saturation state, and only notable within a saturation state treatment. Line 23- but the authors (and others) previously noted that proton pumping is not nearly as energetically expensive as the protein synthesis. Therefore, this argument begins to fall apart again. If there is an energetic basis for calcification, the proton pumping is not the primary energetic cost, (see also work by Cohen on this in corals).

The argument here for the ratio of bicarbonate to proton ion concentration has no more support than a direct saturation state sensitivity noted by Waldbusser et al. 2013, 2014 , in larvae in particular because the calcification surfaces are more exposed to the external environment, as documented by the greater incorporation of DIC C into shell, even though the respiration rate is supposed to be greatly elevated at this stage. At a minimum, the authors should provide the rationale for both if they are arguing for

Page 1556 Line 11 onward. There are a handful of papers that challenge the persistence and presence of amorphous calcium carbonate, it would be best to note those too. Interestingly, a careful read of Medakovic 2000 indicates that the study is not infact noting amorphous CaCO3, the reference is to amorphous tissue, which has been misread and mis-cited in several subsequent papers as amorphous CaCO3. Also most mytilids as adults, produce both calcite and aragonite in varying proportions.

Line 24- this is exactly the kinetic argument made in the Waldbusser et al. 2013 paper, it should be cited here.

1557 Line 4 onward, again, same argument laid out in Waldbusser et al. 2013.

Line 9- There are several papers on this topic, with regards to the isotopic record, which

first shows that seawater DIC is being incorporated to a large degree, that the relative amount of respiratory carbon varies over the life history (increasing with age/slower growth/more food).

Line 15- Waldbusser et al. 2013 also made this argument for C. gigas, and estimated the energetic demand for this early shell formation relative to typical egg lipid reserves.

Line 22- Actually Moran and Manahan (2004) show that egg reserves would be depleted, and along with some previous work, highlight the likelihood of direct DOC uptake as an energy source.

Line 24- Moran and Manahan also did not look at settlement, but Barton et al. 2012, comes really close, in terms of looking at exposure effects during the first 48 hours on the survival of larvae to pedi-veliger stage. This seems like it would support the arguments made here.

Line 25- Waldbusser et al. 2013 did make this argument, but for completion, it isn't just the stress on the energy budget, it is due to the rapid rate of precipitation, which requires significant energy to support as the saturation state approaches undersaturation. In fact, most of the argument laid out in the preceding paragraphs, were noted by this source.

Page 1558 Line 3- check the Hettinger et al. 2013 paper, this does not address feeding per se, but others have, see Vargas et al.

Line 5- So the conclusion is lowered carbonate ion concentration, while several paragraphs were spent arguing for the bicarbonate/proton ratio.

As far as I can tell, you have a different species, but not different populations of the same species. Although the authors state the uniformity in response between these two species, they never state where the oysters are collected from.

Line 8- You cannot take credit for the kinetic idea, without citing the original reference of that work (Waldbusser et al. 2013), these results support that idea, but you counter

this in the discussion with the heavy focus on the proton flux model. What seems odd, is this paper is cited in the manuscript, but not acknowledged for the ideas that have been developed.

End of review

Interactive comment on Biogeosciences Discuss., 12, 1543, 2015.