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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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We thank George Waldbusser for his detailed review on our manuscript and considered his comments in the revised version. We do not agree upon the main criticism, that our experimental design did not allow to answer our specific research questions and that we draw unproven conclusions and respond in the following. The experiments 1+2 were designed in order to generate treatments with low and high [CO32-] independent of the seawater pH and pCO2 which was achieved (see table 1). We decided to replicate our treatments and analyse them by ANOVA instead of generating a matrix to assess the response by regression analysis. All treatments were run with a replication of four and replicate means of the calcification response measured by shell mass growth (juveniles) or shell size (larvae) were plotted against various seawater

carbonate systems parameters. Thereby we obtained a similar response as presented in Waldbusser et al. 2014. We carefully read Waldbusser et al. 2013 and agree on the aspects of high âĎę at the site of calcification and kinetic limitations of crystal formation and better integrated this important hypothesis in the revised version. However, the conditions at the mineralization front are expected to differ considerably from the external medium, even in bivalves which lack extracellular pH regulation. E.g. the extrapallial fluid, which is in close contact to the shell has a substantially different carbonate (or acid-base) chemistry than the ambient seawater (Thomsen et al. 2010, Heinemann et al. 2012). Therefore, we do not think that seawater [CO32-]/âĎę is a valid predictor of calcification as suggetsted by Waldbusser et al. 2014, despite the obvious correlation. Furthermore, the differentiation between seawater [CO32-] and âĎę is not possible as long as [Ca2+] are not changed owing its definition. [CO32-] and âĎę express exactly the same chemical parameter, since âĎę is directly calculated from [CO32-] which makes it impossible to differentiate between these two parameters at similar seawater [Ca2+]. Increasing seawater [Ca2+] is potentially not a valid approach as larvae may react sensitive to increased [Ca2+] in the seawater (Waldbusser et al. 2014). Therefore, the published data are not a conclusive proof of the importance of seawater âĎę. Nevertheless, our results confirm the good correlation of seawater [CO32-]/âĎę and calcification under normal TA conditions (page 1553, line 25). However, calcification is no longer correlated to [CO32-]/âĎę under DIC limiting conditions which questions the general validity of this correlation. Instead we introduced a concept of HCO3- availability and H+ excretion expressed as the ratio [HCO3-]/[H+]. This provides a more physiologically based model explaining the calcification response, considering both the new results obtained in this study and the known mechanisms of membrane transport present in animals with verified transport of HCO3- and H+. Therefore, we introduced the term carbonate equivalents ([CO32-]e) which is, at the same temperature and salinity, directly proportional to the ratio [HCO3-]/[H+] or âĎę. We explicitly stated in the first ms version that we do not expect higher calcification in response to elevated seawater [HCO3-] but only reduced calcification as a result of low, limiting [HCO3-] (page 1555

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line 3+14+16). Furthermore, pH (=[H+]) or [HCO3-] as single factors have no clear effect but only the combination of both (page 1554 line3). Furthermore, the reviewer criticized the normalization of the calcification response to a relative response between control and treatment in the meta-analysis as it potentially masks a further increase of calcification at higher [CO32-]eq (as observed by Waldbusser et al. 2014). We therefore integrated additional data obtained over a wide range of seawater [HCO3-]/[H+] or [CO32-]eq (new Fig. 4D). These data evidence that calcification rates (expressed as shell length) do not further increase at higher [HCO3-]/[H+]. Therefore, the approach used the meta-analyses is an appropriate measure of the calcification response.

Page 1545 Line 8 see general response, We do not thing that these parameters are good predictors but in general many studies simply refer to seawater [CO32-] or âĎę. We agree that the ability to regulate the internal acid-base status potentially compensates the external carbonate chemistry, however, may require adjustment of the cellular energy fractionation (see recent data by Pan et al. 2015 on sea urchin larvae) and thereby may reduce the scope for growth.

Line 10 ‘Strong‘ has been removed.

Line 15 Dickinson et al. 2012 did not measure calcification, Waldbusser et al. 2011 is cited in the revised ms.

Line 25 see general response. ‘seawater‘ has been added.

Line 27 As the carbonate system is a buffer system, its speciation and seawater pH are directly correlated. Therefore it is fair to say that pH effects CO32- and vice versa.

Line 25 see general response, and specific response to comment on Page 1555 Line 23

Page 1546 Line 2 citation added

Line 6 the section on calcification has been modified.

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Line 19 citation added

Line 24 agreed, but the Mediterranean, where M. galloprovincialis originates from, is an example of an ocean area with higher salinity and alkalinity.

Line 29 This refers to pCO2 which is going to increase in variable coastal habitats faster than atmospheric concentrations. Therefore also higher variance of pCO2 can be expected. It is not the case for [CO32-] or pH where the opposite can be expected, due to carbonate system specification.

Page 1547 Line 1 We do not aim to discuss the origin of ocean alkalinity in a final manner but need to mention this topic due to its relevance for calcification of mussels in the Baltic Sea and therefore cited Beldoskwi et al. 2010.

Line 9 citation Waldbusser et al. 2011 added

Line 13 'strong' refers to a broad range of pCO2 (0-4000 μatm) and alkalinity (540-5765 mmol kg-1) treatments. We do not mention the details in this introductive paragraph but give the specific information in the Materials and Methods and table 1.

Line 20 We collected same sized animals Page 1548 line 4. 'M.' changed to Mytilus.

Page 1548 dates of the experiments, replicate numbers, 'filtered' and 'sp.' have been added.

Line 15 corrected to 21 days.

Line 23 reference Thomsen et al. 2013 added

Page 1549 see previous comment, information on animal number and time of transfer added. No polyspermy was observed, which is mostly a problem when using strip spawning.

Line 10 All larvae reached D-stage on day 4 but did not start PD II formation, due to food deprivation. See Suppl. Fig. 2 for the delay of shell formation at day 2. It is not

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clear or us how the same treatment should bias the results?

Line 22 Information on carbonate system sampling and determination protocol are added to the M&M.

Page 1550 Line 4: The calculations were made for M. edulis using data for larvae published by Sprung et al. 1984a and for benthic stages by our own work. Whereas there might be differences between species or even populations, the data by Sprung only comprise one population from the North Sea, the data by Thomsen et al. only specimens from Baltic Sea. Dissolution of shells might have a minor effect on the total calcification rates. However, dissolution rates are at least an order of magnitude lower than calcification of well fed animals and do not play a significant role under low pCO2 (see also Duarte et al. 2015). So far, no data are available that confirmed decreased shell thickness in same sized animals. Previous studies (Gazeau et al 2010, Gaylord et al. 2011) compared smaller high pCO2 treated animals with larger control animals of the same age. As shell thickness increases with size, it can be expected that smaller sized larvae have a thinner shell. In contrast, we have reported similar shell thickness in same sized juvenile animals grown at a pCO2 up to 4000 μatm (table 4, Thomsen et al. 2010).

We have no intention to estimate calcification from respiration rates. The data in Fig. 5a only confirm isotopic data that the inorganic carbon in bivalve shell needs to derive from the seawater DIC since respiration rates are not sufficient to provide enough carbon to calcification, especially during D-shell formation. The respiration and calcification rates of larvae were both determined at 18°C (Sprung 1984 a,b). The meta-analysis in Fig. 5 only depicts the change of calcification and respiration over ontogeny without any direct link to OA and potential impacts on these parameters. This data only highlight the relative importance of calcification and PDI formation in particular compared to the overall animal energy turnover expressed as respiration rates which are a direct measure of energetic requirements.

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Line 21 We did not 'correct' the calcification response as we express calcification relative to an internal control. As we focus on the impact of carbonate chemistry we do not consider potential temperature effects, but again overcome this problem by using a relative response. The good correlation confirms the important effect of the carbonate chemistry. We added additional data to this meta-analysis, see ms for details.

Page 1552 line1 We added the citation Sprung et al. 1984 for temperature effects and refer to our own data for the impact of carbonate chemistry.

Line 12 see general response above.

Line 15 the comma error has been introduced during the type setting. The pH value has been corrected.

Line 18 see response page 1545 line 27.

Line 1553 Line 1 see general response

Line 20 See comment above and also Material and methods section 2.4, respiration rates correlate with shell size as body mass correlates with size.

Page 1554 Our study deals with bivalve calcification.

Line 3 We agree that many physiological processes are affected and calcification is one it.

Line 10 Waldbusser et al. 2013 did not test the impact of carbonate chemistry on bivalves. The reviewer potentially refers to Waldbusser et al. 2014? Which is cited in the revised ms. However, as bivalves cells are characterized by a negative membrane potential, passive proton influx is most probably happening also under low pCO2/high pH. Under these conditions, acid-base regulation may play a minor role as calcification is potentially limited by protein synthesis, see discussion in the revised ms.

Line 17 Waldbusser et al. 2013 is cited in the revised ms.

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Line 21 See general response above and the revised Fig. 4d.

Line 25 Dickinson et al. did not measure calcification. We do not see a realistic scenario in which estuaries with low alkalinity may have a high [CO32-], as long as they are more or less in equilibration with the atmospheric [CO2]. We are aware of the fact, that organic buffer capacity contributes to TA in estuaries, such as the Baltic (e.g. Kullinski et al. 2014), but it does not weaken the statements made in this study.

Page 1555 Line 3 See also general comment above. The reviewer criticizes the concept for calcification of this study, however, does not provide an alternative explanation for reduced calcification at high seawater [CO32-]/âĎę when HCO3- supply is limited.

Line 9 citation Mount et al. 2004 added, but see also comment by Dorrit Jacob. The detailed mechanisms are not understood, which has been considered in the revised version.

Line 14 See general comment, in accordance to our data [HCO3-] is of minor importance as long as it is not limiting.

Line 15 see also general comment and previous comment, for a given salinity and temperature, carbonate system manipulations result in a proportional change of [HCO3-]/[H+], [CO32-] and âĎę.

Line 23 in the revised ms, we discuss the importance of H+ pumping for total energetic costs at control compared to acidified conditions.

As mentioned above, our results support the apparent correlation of seawater [CO32-] / âĎę and calcification for normal seawater conditions, but the correlation is not visible under DIC limiting conditions. This speaks against the importance of seawater [CO32-]/ âĎę. In particular the conditions in extracellular fluids differ substantially from the ambient conditions as pointed out in this review. We do not argue against the importance of âĎę in the calcifying fluid (in line with Waldbusser et al. 2013), but question the importance of the seawater âĎę as suggested in Waldbusser et al. 2014. The

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hypothesis that the calcification surface of larvae is more exposed to seawater than in juvenile is not supported by any data. Our data reveal similar responses for larvae and juveniles; higher tolerance of benthic stages might result from relatively lower calcification rates or less energy limitation. The higher fraction of metabolic CO2 in carbonate shells as observed by isotopic measurements may only represent the different ratio of respired CO2 to calcified carbon as also shown in Figure 5a. Consequently relatively more metabolic carbon may passively diffuse into the calcifying fluid. This aspect is briefly discussed in the revised ms.

Page 1556 Line 11 We cited Medakovic 2000 as it states an aragonitic shell in larvae. The other citations refer to amorphous calcium carbonate. The first shell of juveniles is made out of calcite before the secondary thickening starts by aragonite formation.

Line 24 Waldbusser et al. 2013 is cited in the revised ms.

Page 1557 line 4+9 Waldbusser et al. 2013 refers to isotopic data which indicate higher use of seawater DIC compared to metabolic data. Our data are based on the direct measurements made by Sprung 1984 a,b which are discussed in this section. The results of both approaches are similar; consequently Waldbusser is cited at the end of this paragraph.

Line 15 Our citations refer to original publications on the onset of feeding in bivalves.

Line 22 we added this aspect in the revised ms.

Line 24 Barton et al. 2012 and Thomsen et al. 2010 added and discussed.

Line 25 we acknowledged the work by Waldbusser et al. 2013 in the paragraph.

Page 1558 line 3 this citation was added in order to discuss compensatory effects of food supply not potential disturbances of food uptake or digestions (see e.g. Stumpp et al. 2012).

Line 5 In the revised ms, we clarified the similarities and differences between our con-

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cept and those published by the reviewer.

Line 8 We cite Waldbusser et al. 2013 in the revised ms.

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