

Interactive comment on “Calcification in a marginal sea ndash; influence of seawater [Ca²⁺] and carbonate chemistry on bivalve shell formation” by Jörn Thomsen et al.

Anonymous Referee #2

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General comments:

This study addresses the impact of calcium ion concentration on first shell formation in bivalve larvae (Mytilid mussels) independent of salinity and in concert with changes in seawater carbonate chemistry associated with ocean acidification. The impacts of changing seawater chemistry on the biology of marine calcifiers has been the topic of extensive study in the last decade particularly with respect to changes in pH and carbonate chemistry under current and projected changes in atmospheric CO₂ concentrations. The current study addresses a potential compounding factor-low levels of calcium ions for calcification and shell formation in brackish waters of the Baltic Sea.

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The approach employed combines 1) evaluation of biological response (capacity for first shell formation, ion concentrations in the extracellular calcification space) under experimentally manipulated calcium ion concentrations alone, and in connection with altered carbonate chemistry and 2) a comparison of the experimental findings with environmental conditions (and variation) in the Baltic.

The experimental approach was appropriate and well executed. I appreciate the efforts to address biological responses at the level of the whole organism as well as components of physiological response (ion concentrations). Further, the synthesis of environmental data contextualizes the biological responses for discussion of their implications.

I have included below several questions and comments that may guide the authors in refining and clarifying their presentation of the study.

Specific comments:

-Is shell length along a sufficient assessment of the impact of seawater chemistry on calcification? Using shell length as the metric for calcification assumes that a relationship between length and mass of the shell is consistent over different environmental treatments and there are indications this is not the case (Frieder et al 2017 ICES JMS, Gaylord et al 2011 JEB).

-It would be helpful to clarify how “larvae that had not developed a complete PD I shell” were assessed. Does this mean shell was abnormal? Partially developed? Would they have developed given more time, or a change in conditions? For instance, at Line 263, were the 7 day old larvae with a 63.7 μm shell diameter just small versions of a complete PD I?

-As presented, I don't find the suggestion that the “troussulus-like” animals have evolved higher tolerance for low calcium any more compelling than the possibility that they are acclimatized to the prevailing conditions experienced at the collection site. The

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suggestion is of course interesting based upon East-West gradient of allele frequencies from Stuckas et al. However, in the absence of any data to validate that the broodstock collected from the different sampling sites are genetically distinct adaptation doesn't seem to be more or less favored over a plasticity argument.

-Can any comment be included on the historical distribution of these populations suggesting that they have expanded/contracted?

-I am having a hard time interpreting figure 3B. CS calcium continues to decline with the reduction of seawater calcium, but it is no longer following seawater. You propose that this is a surplus of calcium stored up when shell formation rate plummets? So, some other component of the calcifying process is inhibited at this level, and must be limiting the utilization of this calcium?

Minor comments/corrections:

-Line 120-"Finish" should be "Finnish"

-Fig 3 is introduced in the results before Fig 2b,c,d. Further, the paragraph (starting at line 271) regarding results of microelectrode measurements should probably be the last the paragraph of section 3.1. This would move the current, last paragraph of the section (which contains reference to Fig 2bcd) into the appropriate presentation order.

-Line 280-reference to Figure 1, should be Figure 2

-It would be helpful to indicate the control treatment levels on Fig 2 since higher and lower [Ca²⁺] treatments are applied

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