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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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Thomsen et al. report on an interesting study designed to gain insight into which was the efficient carbonate species upon calcification in two types of bivalves including their larvae. The biomineralization of molluscs and particularly bivalves is subject of intense study and our knowledge in this area may seem relatively advanced. However, the recent paradigm change, which acknowledged the universal importance of stepwise crystallization pathways involving transient precursor phases (e.g. ACC), has raised abundant new questions on the mechanisms that lead to the formation of bivalve shells. In the light of this emerging strain of research, studies such as the one by Thomsen and co-workers that straddle the disciplines are critical and drive progress in our mechanistic understanding of biomineralization. At the same time, interdisciplinary studies

contain their own specific pitfalls and I would like to comment here on the presentation of the current knowledge in biomineralization of bivalves, which I find rather too simplistic. These issues are admittedly marginal to the main messages in this manuscript, but appear to me worth commenting on in order to highlight the current debate under the umbrella of an interactive scientific discussion promoted by the journal.

I am referring particularly to the two statements below:

p. 1546, l. 6ff. "Calcification involves intracellular production of an amorphous calcium carbonate (ACC) precursor which is exocytosed from the calcifying epithelia and transported to the site of shell formation (Weiner and Addadi, 2011). The precursor is then integrated into an organic matrix framework and remains either transiently in the amorphous state or crystallizes into a specific polymorph such as aragonite or calcite depending on the specific properties of matrix proteins (Weiss et al., 2002; Jacob et al., 2008)."

p. 1555 l 7ff. "Intracellular calcification requires a concentration mechanism for Ca2+ and HCO3- in specialized membrane enclosed intracellular vesicles to produce the amorphous calcium carbonate (ACC) precursor (Weiner and Addadi, 2011)."

Unfortunately, our current state of knowledge on bivalve biomineralization is by no means as clear and uniquely accepted as these statements may imply. While it seems a fair extrapolation that ACC is widespread in larval calcification (Weiss et al., 2002, cited in the ms), direct evidence for ACC in adult bivalve shells is currently only available for Unionoida (Jacob et al., 2011, not cited in the ms). We simply do not know how C. gigas and Mytilus "do it"; common sense may argue for a crystallization pathway using ACC, but there is currently no evidence at all. The same holds for the vacuolization and transmembrane transport mechanisms of the carbonate species to the site of mineralization in bivalves. Evidence for the existence of "specialized membrane enclosed intracellular vesicles" as summarized in Weiner and Addadi (2011), referenced in the ms stems from studies mainly in the 1970's that were neither always targeted at the

mineralizing tissues nor at bivalves alone. For example, “Ca-bearing granules”, are also reported from insects, clearly without connection to calcification in bivalves. While I do not want to discredit any of the older studies, the findings of “Ca-bearing granules” are yet to be reproduced with state-of-the-art methods targeted specifically at the mineralizing tissues in bivalves. This is currently at the very edge of what is analytically possible, but has successfully been carried out for Amorphous Calcium Phosphate (ACP) vesicles in zebrafish (Mahamid et al., 2013).

I think the above highlights some of the complications quite well and I would wish that the revised version of the ms reflects this a little more.

Jacob, D.E., Wirth, R., Soldati, A.L., Wehrmeister, U., Schreiber, A. (2011). Amorphous calcium carbonate in the shells of adult Unionoida. Journal of Structural Biology, 173, 241–249.

Mahamid, J., Aichmayer, B., Shimoni, E., et al (2010) Proc. National Acad. Sci. USA, 107, 6316-6321.

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