

Interactive comment on “Inter- and intra-specimen variability masks reliable temperature control on shell Mg/Ca ratios in laboratory and field cultured *Mytilus edulis* and *Pecten maximus* (bivalvia)” by P. S. Freitas et al.

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General comments

In general, I liked this discussion paper and the overall scientific quality is quite good. Nevertheless, I have quite a lot of points I want to stress and several recommendations. They are in the specific comments.

I find the analytical methodology really not clear enough.

In general, I find the paper quite hard to read. Some recapitulative tables could help

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(but see below).

Specific comments

Major points

Oxygen stable isotopes: First of all, I will remove the whole part(s) concerning d18O because this is not the point here. These results do not support the problematic raised by the authors. In addition, there is only one sentence in the discussion talking about these results, L 1-2 p. 550; which, in my point of view, is irrelevant. Nothing in the abstract or conclusion concerning d18O so clearly must be removed unless more discussed. If the authors still want to keep these results, I would like to know which d18Oseawater they used when they talked about "d18Ocarbonate minus d18Oseawater". Do they used a mean d18Oseawater for all points or used mean d18Oseawater per growth period? As d18Oseawater was regularly measured, precise d18Oseawater can be used for each analytical point.

Shell ages: Are the specimens used not all juvenile? Something should be said about that as differences might be observed in shell chemistry before and after sexual maturity. Should be discussed somewhere.

Environmental parameters:

L 11-13, p. 536 it is said that "animals of similar size were moved into separate aquaria each under different but constant temperatures and controlled food and light conditions". If temperature and food conditions are described, this is not the case for light. What do you mean by "constant light conditions"? Not discussed further.

Concerning food, it is said that a constant quantity of food was supplied to the specimens but what about possible food supply via the natural seawater pumped into the aquariums?

The possible (or not?) influence of food intake, that is most probably different from animal to animal, on shell Mg incorporation is not discussed. That could be an additional

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hypothesis to explain the discrepancies observed (at the different levels).

As natural seawater was supplied, what about possible Mg/Ca water content changes? If no important freshwater inputs are observed in the region, should just be said somewhere that Mg/Ca seawater content is constant.

Nothing on possible water pH changes and potential influence on shell Mg/Ca ratios.

Analytical points:

I find the analytical methodology description hard to follow or even not clear at all (p. 542).

L 3: You talk about "synthetic standard solution in the range of 0t25 mmol/mol for Mg/Ca". Apart of the "t" in "0t25", which is probably a comma in fact, you are not giving a "range" but a single value and I wonder why you are giving a ratio (Mg/Ca) rather than a Mg concentration, as you do for Ca.

What are the "N" you are giving into parenthesis (and this question is valuable all along the paper)? Do you mean you analyse 304 std solutions (so, much more than the number of your shell sample)?

L 5: "The smallest milled powder samples were analysed at 30 μ g/ml"; 30 μ g/ml of what? Of Ca? So less than your less concentrated std solution?

L 8: The intermediate calibration standard is at "16 mmol/mol" of Mg/Ca (not specified). Either you specify at the beginning of the paragraph that all data in mmol/mol will stand for Mg/Ca ratios or you must specify each time.

L 9: "and data then were corrected accordingly" Which means? What percentage of derive was set as the maximum authorized?

"Analytical precision (expressed as relative standard deviation or RSD) was 0.5% for the laboratory cultured specimens (N=86) and 1.3% for the field cultured specimens (N=29)." How was this analytical precision calculated? Are these RSD the ones from

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different replicates of each sample analyses (e.g. mean of 3 replicates per unknown solution)? Why is this analytical precision different between laboratory and field specimens?

"In the laboratory culturing experiments, sufficient material was not available from any one growth interval to enable replicate analyses for an assessment of true sample precision; in the field experiment, however, sample precision was better than 6.2% RSD for replicate measurements (N=3) of the same milled powder samples obtained from five *M. edulis* specimens." Can be cut into two sentences and not clear enough. If I understand well (still, we must guess), you managed to have one large powder sample that allowed you 3 replicate measurements but 3 replicate measurements of the same solution or do you split you powder into 3 (which is probably what you have done)? And you obtained such large sample for 5 shells so you have 5 measures of the true sample precision? That's it? If this is 3 replicate measurements of the same solution (I don't think it is), 6.2% is quite a "bad" value. If this is analyses of split milled samples, this value is understandable.

As this part is already confused, I would remove the results concerning the ongoing international calibration study (even if I can understand that the authors wish to get those data published). In addition, these results are of course not discussed. That will also remove a table. For me, these results are unnecessary here.

You referred to Freitas et al. 2005, 2006 for accuracy but that would be nice to specify here that accuracy.

In fact, a kind of summary of the analytical precision and accuracy (table or even just a final sentence?) would be nice also.

In Figure 6 and 7, you talk about twice the analytical precision which corresponds to 0.1 mmol/mol for Mg/Ca, why not give that in the text?

I would find a kind of recapitulative table, with mean Mg/Ca results from that study

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and those from previous work, really helpful for the reader, particularly to follow up the discussion. That would also allow the authors to reduce the text somehow.

Same thing could be done for regressions (quite heavy in the text).

Other points (in the order of appearance in the text)

L 20-21, p. 533: The references cited are all about foraminifera and corals. Is it not possible to add a reference on bivalves?

L 24, p. 538: "each shell was identified by a mark hand drilled on its surface" Didn't this handling involve stress in the animals? Is there something identifiable at the shell surface, for shell secreted after this drilled mark?

L 18-19, p. 539: I do not understand what you mean by saying that: "by assuming shell growth rate to be constant during each growth interval" here.

L 11, p. 540: "depth and width of milling were controlled carefully": be more specific please.

L 23, p. 540: "for measurement purposes": It is said L. 17-19, p. 537 that "Each time the *M. edulis* specimens were removed from the aquaria they were exposed to the air for 5 to 6 h" Five to six hours for "measurement purposes"?

L 3-7, p. 541 (last sentence before § 2.3.2.): So you mean that the disturbance marks where not sampled? Specify if this was intentional (I guess so) and why.

L 14-15, p. 541: "On such occasion..."; not clear to me.

Title 2.4, p. 541: I would change it by "Mg/Ca analyses" (or at least change "elemental ratio" by Mg/Ca).

L 8, p. 545: Why not give a mean (all analyses species by species for each given temperature) ± 1 sigma;? That will be clearer.

L 16-18, p. 545: I do not find the statistic results for significant differences for Mg/Ca

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ratios between experiments two and experiment one. And are Mg/Ca ratios between experiments one and two really significantly different for around 12 and 15°C? No so evident on Fig. 6a, particularly for 12°C for which Mg/Ca ratios seem similar for both experiments. And not discussed.

L 7-8, p. 546: This sentence is a repetition of the L18, p. 545.

L 7-8, p. 549: "i.e. between milled samples...shell level)." You want to say for the monitored laboratory experiment at a constant water temperature here? Because otherwise Mg/Ca changes could be related to water temperature changes (in the field experiment).

L 26-27, p. 549: What are the N here (because it refers to the Freitas et al., 2006 work)? Where can we find in the Freitas et al., 2006 study the "Mg/Ca ratios varied by up to 4.06 and 5.61 mmol/mol"? On their Fig. 8?

L 29, p. 549: Mg/Ca ranges for field-cultured *M. edulis* shells (this study) are given (start of § 3.3) but intra-individual variability are not given as numbers, that complicates the comparison with previous data given in the former § p. 549. Also, former studies results are given as "Mg/Ca ratios differences". I found these ratios for the present study for the laboratory shells only.

P 550: Grey points on Figure 8 are not discussed (and are not specified in the figure caption also).

L 15-16, p. 553: "with only a weak correlation for *P. maximus* ($r^2=0.21$, $p<0.001$)." A word could be added on the relationship observed between *P. maximus* Mg/Ca ratios and salinity by Freitas et al., 2006.

L 10-22 p. 554: Background on small scale Mg heterogeneities in *M. edulis* shell is given here. But the points reported in that paragraph are not discussed in relation with the present study. So, either the authors add some discussion or that paragraph can (must?) be omitted.

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Technical corrections

L 19, p. 534: in the parenthesis citing "Klein et al., 1997; Immenhauser et al., 2005"; I would add an "e.g." at the beginning, because there are other.

L 24, p. 534: is "M. edulis. edulis species" correct? (Twice "edulis")

L 13, p. 541: I would add a comma after "permitted".

Title 3.2; p. 545: change "calcite mg/ca" by "calcite Mg/Ca"

Equation (2), p. 546: parenthesis missing. "N" still undefined.

L 15, p. 547: Shouldn't the authors refer to the figure 6b here rather than on figure 6 in entirety?

L 11, p. 552: "This consideration thus prohibits", why "thus" here?

Figures:

Figure 1: Can you increase the police points for the names?

Figure 2: Same thing. T1; T2, T3, M really smalls.

Figure 4b: Two shells from the short-deployment experiment were analyzed. Should be indicated in the figure caption (only one curve).

Figure 6b: Why not reduce the Mg/Ca scale (from 0 to 10 mmol/mol), because we can not see anything like that! I do not understand "Each point represent...individual growth interval".

Figure 7: On the graphs, "± 1 sigma" unreadable. And, is it not 2 sigma? (in the caption, you said "twice the analytical error"). The ± 0.1 mmol/mol analytical error is not clearly stated in the text.

Figure 8b: No reference for the grey points (M. edulis; Menai Strait). What is the dashed line? As individual points of the Vander Putten et al., 2000 study are not shown, why

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not display their results as a light shaded area? Legend of the points on the figure (8a and 8b) quite small.

References

L 27, p. 556: change "Debeney" by "Debenay"

L 17, p. 557: "Elferfield et al. 2002"; cited in the text as "Elderfield et al. 2001" (L 10, p. 534)

L 21, p. 557: "Erez et al., 2003"; cited in the text as "Erez et al., 2005" (L 14, p. 534)

L 1, p. 558: "Gardner et al., 1992"; change page number from "219-213" by "219-243"

L 12, p. 559: change "Papanicolau" by "Papanicolaou"

L 9, p. 560: change "Oxyegn" by "Oxygen" (twice)

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