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Biogeosciences Discussions

Interactive comment on “The influence of temperature and seawater carbonate saturation state on ^{13}C-^{18}O bond ordering in bivalve mollusks” *by* R. A. Eagle et al.

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The influence of temperature and seawater carbonate saturation state on 13C-18O bond ordering in bivalve mollusks. Biogeosciences Discuss. 10, 157-194, 2013.

Authors response to reviewers.

Here we present responses to comments from Anonymous Referee #1 and Stefano Bernasconi. As the main comments from both reviewers concern the same issue we combine our response to these comments below. Reviewer #1 also had a number of minor comments, which we respond to below our response to the main comments of both reviewers.

Anonymous Referee #1 (Main Comment):

I have one major (?) concern and a handful of minor and technical comments. My concern has to do with the robustness of the derived slope, given the size and scatter of the new dataset. The authors mention that this dataset substantially expands existing data from modern mollusks (which it does), and they also explore the effect of removing from the regression several samples grown/collected at low temperatures. However, both with and without the low-temperature data, there is appreciable scatter in the mollusk data. In my opinion, this decreases confidence that the authors are, in fact, observing a slope significantly different from that of Ghosh et al. (2006) and the biogenic carbonate compilations.

Reviewer #2 Stefano Bernasconi (Main Comment):

The main problem I have is that I am not convinced that this dataset is really significantly different from the data of the Ghosh and Tripati calibrations, as concluded by the authors. Indeed when analyzed alone the obtained regression has a much shallower slope than the more extensive Tripati dataset. However, when plotted all together in the same diagram, (Figure 1, plotted from the data given in the tables and supplementary information), we observe that most new data overlap very well with the Tripati dataset, with only a one cultured and two natural samples sample at 10_C and two field samples at 0_C that have a significantly lower D47 and a couple of samples with higher D47 at 25_C. All other samples, hovever are essentially within the scatter of the Tripati data (Fig 1). When all data are plotted together, (figure 2) we obtain a temperature dependence of 0.0476‰

$_{C} whichislowerthantheTripatietal.studybutstillhigherthanwhatisobtainedbythemolluskdataalone.Alsothisfiguredoesnot$
$325.), IthinkthatthepossibilitythatthesenewdatajustconfirmthepreviouscalibrationoftheCALTECHlabshouldbediscusse$

Author response:

Both reviewers highlight important issues relating to how well both our study and previous studies have really constrained the slope of calibration lines, how much variation

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(biological/ methodological/mineralogical) is present in the dataset and what effect that has on calibrations, and what extent calibration slopes could be biased by the inclusion/exclusion of certain samples. To some extent we feel we did anticipate these issues in our original submission, for example in Section 3.3 we did present analyses of what effect excluding certain samples (namely mollusks from the coldest environments at Antarctica and one apparent outlier, the taxa Z. patagonica) had of the slope of regression line. We do note, as Reviewer 1 does in their review, that this exclusion of these samples does make the slope slightly less shallow (page 173 line 1 of the discussion paper). Additionally we do present a number of statistical tests in Sections 3.2 to 3.4 to demonstrate whether the slopes of different regression lines through our data and published data are statistically different (pages 169-173). For example from these stats test we are able to make the statistically robust conclusions that a regression line though all our mollusk data is significantly shallower than the original inorganic calibration of Ghosh et al., 2006, and that there is no statistically significant different between calcite and aragonite in our dataset (see section 3.4 of our discussion paper – page 173, line 13). Nevertheless as both reviewers highlighted this as an area an area that they would like to see more work done in our manuscript we have made a number of modifications to expand on these points and address the areas the reviewers highlighted:

1. Added analysis in the text to compare how the residuals (variation from expected values) of our mollusk dataset compare to another datasets comparable is size, the calibration of foraminifera of Tripati et al., GCA, 2010 mentioned by reviewer Stefano Bernasconi, and deep-sea coral by Thiagarajan et al., GCA, 2011. Specifically both reviewers have a perception that the mollusk data has a level of “scatter” that makes the slope of regression lines uncertain, and so we analyzed whether the residuals from mollusk dataset were greater or similar to those of previously published datasets. 2. Including a comparison (including stats tests) of our mollusk data and subsets of the mollusk data to the biogenic compilation of Tripati et al., 2010 (with the coral data of Thiagarajan et al., 2011 also included), whereas before our analysis had mainly

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focused on a comparison between our data and the inorganic calcite calibrations of Ghosh et al., 2006 and Dennis and Schrag, 2010. In the revised manuscript we will include a new Table (Table 5) that presents the results of statistical analysis (ANCOVA) of the mollusk linear regression lines and subsets of the mollusk datasets compared to inorganic calcite calibration as well a compilation of previously published biogenic data from our laboratory.

For point 1 our additional analysis suggests that the reviewers intuition that there is more scatter in the bivalve mollusk calibration than other previously published studies is supported by a comparison to other biogenic calibration datasets produced in our laboratory. The R2 value of our mollusk linear regression is 0.7258 and the standard deviation of the residuals (SDR) from this line are 0.017. This compares to an R2 value of 0.8998 and a SDR of 0.014 for the foraminifera calibration data of Tripati et al., GCA, 2010 and an R2 value of 0.8703 and SDR of 0.015 for the study of corals presented by Thiagarajan et al., GCA, 2011. Therefore we have added the following text to section 3.2 the manuscript:

“The R2 value of our bivalve mollusk calibration line is 0.0728 (Table 4) using data on the absolute reference frame, and the standard deviation of the residuals (SDR) is 0.017‰Ṫhis suggests that there is somewhat larger variability in bivalve $\Delta 47$ data compared to other biogenic calibration datasets. For example the linear regression through the foraminifera calibration of Tripati et al. has an R2 value of 0.8998 and a SDR of 0.014, and for the study of corals by Thiagarajan et al. the R2 value is 0.8703 with a SDR of 0.015 (Tripati et al., 2010; Thiagarajan et al., 2011). It is possible that this reflects very subtle biological or mineralogical effects on bivalve $\Delta 47$ data, although as we describe below we cannot resolve these effects in our dataset.”

We do note however that whilst the reviewers are correct that the mollusk calibration has more variation in it than other datasets, this variation does not affect the validity of the statistical analysis of the difference and similarity of calibration slopes we present in our manuscript and in Table 5 attached as the statistical analysis does take into

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account the variability in each dataset being compared. Therefore in Section 3.3 of a revised manuscript we will add the following text:

"We also note that the apparently higher variability in the bivalve mollusk dataset compared to other biogenic calibration datasets is taken into account by the statistical analysis of slopes presented in Table 5 and so this variability itself cannot explain the statistically significant differences in slopes we observe."

On point 2 we show in the new Table 5 in our revised manuscript (also attached here) that a statistical analysis of slopes indicates that our mollusk dataset is statistically different from both the inorganic calcite calibration of Ghosh et al., GCA, 2006 and the compilation of previously published biogenic data produced in our laboratory. We also add some additional discussion of Table 5 to section 3.3 and 3.4 of the revised manuscript. Therefore we feel we must stick with the conclusions that we advanced in our discussion paper that this mollusk clumped isotope calibration has a different slope to previously published datasets from our laboratory.

To further address point 2 we have modified the original Table 4 to include linear regression analysis on the bivalve mollusk calibration dataset if specimens from the coldest environments in Antarctica were excluded (in order to assess the potential affect these samples have on the linear regression slope as these specimens are the most different from previously published). However as we show in the new Table 5 (above), exclusion of these data points does not alter the conclusions from statistical analysis of our slopes; that they are different from both the previously published biogenic data from the Caltech lab and the inorganic calcite calibration.

We should also make a number of general observations on these points. Firstly it is correct to say that exclusion of certain samples - perhaps most notably five individuals of aragonitic and calcitic mollusks that grew in very cold (approx. -1°C) environments – does result in a steeper slope. This is a point worth making - as we did in the original text and now also in a revised Table 4 and new Table 5 – however it is also important to point out that at present we do not have a good justification for excluding these data in this way, and this is particularly true given one major area clumped isotope temperature estimates from mollusks will be used for is to study polar climates in the past. As we cite in the main text, previous research on these cold-water mollusk taxa has not revealed evidence for significant “vital effects” on their δ18O values and so we don’t at present have reason to believe they may have vital effects on the Δ47 values. Additionally we note that we have analyzed 5 individuals (each with 2-5 replicate analysis) of these cold water mollusk taxa, consistently finding Δ47 values of 0.72-0.74‰ (on the Caltech Intralab reference frame; see manuscript Table 3), substantially lower than the expected values of 0.80‰ predicted if they conformed the inorganic calcite calibration of Ghosh et al., GCA, 2006. Of course future work may resolve this issue further, but we do not think deviations of this size from the inorganic calibration can be ignored. We have added text to the main text to make these points.

We agree with the reviewers that there is scatter in the calibration data, and it is possible that this is indicative of small biological and/or mineralogical affects. However we do not yet have definitive evidence for these effects and so at present we cannot see a justification for doubting the shallower slope we have observed. We note that the mollusk calibration dataset we present is at least as large or larger in terms of specimens and analyses as previously published calibration studies (eg. Tripati et al., GCA, 2010; Thiagarajan et al., GCA, 2013) and comprises significantly more analysis than the inorganic calcite calibration studies (Ghosh et al., GCA, 2006; Dennis and Schrag, GCA, 2010) so we feel it is justified to analyze the slope of the mollusk data independent from other datasets, rather than binning them all together. Also we feel that we have to be guided by statistical analysis and so if in this case our ANCOVA tests are telling us that the slope of the mollusk calibration is significantly shallower than the inorganic calcite calibration and the compilation of previously published biogenic data from our laboratory we feel we have to go with this conclusion. Finally we have also made note in our revised manuscript of another study that was published after our discussion paper appeared online that also reports a shallower slope for a calibration of brachiopods

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and mollusks (Henkes et al., GCA, 2013). Therefore this study tends to support our observation of a shallower slope. We add the following text to the discussion section of our manuscript:

"We also note that after this manuscript was published as a discussion paper another study of brachiopods and mollusks in a different laboratory also reported a similarly shallow slope (Henkes et al., 2013), although as these measurements were conducted using a very similar methodology to that described in the study presented here the similarity between our calibration slopes does not entirely resolve the possible methodological differences between calibration studies described below."

As we discuss at length in our manuscripts discussion section there are a number of possible reasons for these different clumped isotope calibration slopes, which could include methodological differences as well as small biological or mineralogical affects on isotopic composition. It will take careful work in the future to tease out these possible explanations.

Anonymous Referee #1 (Minor Comments):

Author response: Referee #1 had a number of minor spelling and grammatical corrections which have been addressed in our revised manuscript.

Minor comments: C62 1. p 160, line 5: Comma missing after 'for example'. 2. p 161, lines12-13: The issue is not that the taxa deviate from the fluid _18O - this is, of course, expected. The issue is that they deviate from the _18O they are expected to have, given the _18O of the fluid and their growth temperature. 3. p 161, line 25: The word 'material' should be plural. 4. p 161, line 27: Comma missing before i.e. 5. p 162, lines 6-7: The 'e.g.' should be placed inside the parentheses, followed by a comma. This error appears several other times in the manuscript (see below and possibly other occurrences). 6. p 162, lines 17-21: The first sentence in this paragraph is long and awkward. Please consider rewriting it in short, clear sentences. 7. p 166, line 5: The phrase 'will vary' should probably be 'varies'. 8. p 166, lines 10-13: The sentence starting with 'Water temperatures' is grammatically incorrect. 9. p 168, line 10: There are some extra words in the sentence ('as was the'?). 10. p 168, line 13: Comma missing before, 'which'. 11. p 170, lines 4-9: This sentence is grammatically incorrect. 12. p 170, line 21: Missing 'by' between the words 'confirmed' and 'analysis'. 13. p 170, line 22: The word 'call' should be 'calls'. 14. p 170, line 24: A new sentence should be started with the word 'therefore' (end of the line). C63 15. p 170, line 25: The words 'a summer months' should be 'the summer months'. 16. p 170, line 26: The word 'seam' should be 'seem'. 17. p 171, lines 13-15: The phrase 'the difference between these two slopes ... is not significantly different...' should probably be 'the difference between these two slopes ... is not significant...' or 'these two slopes are not significantly different...'. 18. p 172, line 4: Missing 'do' between the words 'and' and 'not'. 19. p 172, line 14: The word 'effected' should be 'affected'. 20. p 172, line 18: 'carbonate as for example the rate' should be 'carbonate, as, for example, the rate'. 21. p 172, line 25: The word 'to' is missing between the words 'order' and 'assess'. 22. p 172, lines 27-end: Awkward wording. Please consider ending the sentence with the reported slope and intercept and starting a new sentence along the lines of 'This slope is slightly steeper, but within the 95% confidence interval...'. 23. In several places in the manuscript, verbs related to the noun 'data' are singular. They should be plural. 24. p 174, line 24: The word 'less' should be 'fewer'. 25. p 175, lines 5-7: Two 'between' in the same sentence. 26. p 175, line 28: See comment 5. 27. p 176, line 3: The word 'effect' should be 'affect'. 28. p 176, line 9: The word 'revolve' should be plural. C64 29. p 176, lines 14-18: This sentence is awkward and difficult to understand. 30. p 176, line 24: Please consider adding 'This is' before the words 'In contrast'. 31. p 176, line 25: See comment 5. 32. Figure 1 caption: In the second to last sentence, the word 'thank' should be 'that'. A pox on autocorrect! Interactive comment on Biogeosciences Discuss., 10, 157, 2013. C65

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/10/C1510/2013/bgd-10-C1510-2013-

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Table 4. Slopes and intercepts of linear regressions through Δ_{47} and temperature data for samples with known growth temperatures.

	Relative to the stochastic distribution					Absolute reference frame				
Dataset	**Slope**[e]	**1 s.e.**	**Intercept**	**1 s.e.**	R^2	**Slope**[e]	**1 s.e.**	**Intercept**	**1 s.e.**	R^2
Inorganic calcite[a] Ghosh et al, 2006	0.0598	0.0094	-0.0248	0.1046	0.8896	0.0620	0.0099	0.0021	0.1095	0.8877
Inorganic calcite[a] Dennis and Schrag, 2010	0.0316	0.0036	0.2697	0.0382	0.8587	0.0340	0.0038	0.3155	0.0408	0.8600
Published biogenic data compilation[b]	0.0550	0.0019	0.0267	0.0223	0.9140	0.0559	0.0019	0.0708	0.0232	0.9105
All bivalve mollusks This study	0.0341	0.0041	0.2719	0.0496	0.7246	0.0362	0.0044	0.3140	0.0527	0.7258
Bivalve mollusks minus Antarctic specimens[c] This study	0.0378	0.0050	0.1488	0.0601	0.7094	0.0402	0.0054	0.2686	0.0638	0.7098
Calcitic bivalve mollusks This study[d]	0.0342	0.0054	0.2725	0.0658	0.7685	0.0364	0.0058	0.3140	0.0706	0.7656
Aragonitic bivalve mollusks This study[d]	0.0383	0.0074	0.2094	0.0893	0.8180	0.0407	0.0078	0.2483	0.0095	0.8179

[a]See Table S1 for the data used for these regression lines calculations.
[b]Includes coral data from Ghosh et al., 2006 (but excludes Red Sea *Porites*), and data from Ghosh et al., 2007; Came et al., 2007; Tripati et al., 2010; Eagle et al., 2010; Thiagarajan et al., 2011. See Table S1 for values for these data.
[c]Excluding data from the five individuals of *Laternula ellipica* and *Adamussium colbecki* (which are Antarctic specimens from the coldest environments sampled in this study) as a means for determining whether the calibration slope could be significantly influenced by these samples alone.
[d]Excluding specimens with mixed mineralogy
[e]Linear regressions through previously published data are all recalculated here using GraphPad Prism software (Zar, 1984) so that they are directly comparable to the new mollusk data presented here, and as a result may have slight differences from the slopes and intercepts given in original publications at the third or fourth decimal place. All regressions are on data that include an acid digestion temperature correction where appropriate (Passey et al., 2010). Errors are given as 1

Fig. 1. Revised Table 4

Table 5. ANCOVA p-values derived by comparing linear regressions through the dataset generated in this study to previously published data.

Dataset[a]	Inorganic calcite Ghosh et al, 2006	Inorganic calcite Dennis and Schrag, 2010	Published biogenic data Compilation[d]
All bivalve mollusks, this study	$p = 0.0035$ (Y)	$p = 0.7020$ (N)	$p < 0.0001$ (Y)
Bivalve mollusks minus Antarctic species[b] This study	$p = 0.0139$ (Y)	$p = 0.5453$ (N)	$p = 0.0006$ (Y)
Calcitic bivalve mollusks this study[c]	$p = 0.0196$ (Y)	$p = 0.9354$ (N)	$p = 0.0013$ (Y)
Aragonitic bivalve mollusks this study[c]	$p = 0.1274$ (N)	$p = 0.4664$ (N)	$p = 0.0126$ (Y)

[a]Linear regression lines through different subsets of our mollusk Δ_{47} calibration dataset in the first column are statistically compared to using analysis of covariance (ANCOVA) tests (Zar, 1984) to linear regressions through other previously published calibration studies datasets. Calculations are done with values on the absolute reference frame (ARF). The table displays the ANCOVA p-value and whether the two slopes being compared are statistically different; (Y) = Yes, (N) = No. In this case we consider a p value < 0.05 as indicating statistically significant differences between the two slopes.
[b]Excluding the five specimens of *Laternula ellipica* and *Adamussium colbecki* (which are specimens from the coldest Antarctic environments) as a means for determining whether the calibration slope could be significantly influenced by these samples alone.
[c]Excluding specimens with mixed mineralogy
[d]Includes coral data from Ghosh et al., 2006 (but excludes Red Sea *Porites*), and data from Ghosh et al., 2007; Came et al., 2007; Tripati et al., 2010; Eagle et al., 2010; Thiagarajan et al., 2011. See Table S1 for values for these data.

Fig. 2. New Table 5

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