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Interactive comment on "Size-dependent response of foraminiferal calcification to seawater carbonate chemistry" *by* Michael J. Henehan et al.

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Reply to Anonymous Referee comment 2 on **"Size-dependent response of foraminiferal calcification to seawater carbonate chemistry"** ***by*** **Henehan, Evans et al.**

We thank the reviewer for some useful and constructive comments. Our responses to each are outlined below.

Comment: *"In this manuscript, the authors use a combination of laboratory culture experiments, plankton tows, fossil shells and modelling to examine drivers of size normalized weight in foraminifera. They focus efforts on the species* G. ruber *, and identify*

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the importance of shell size and chamber number as predictors of size normalized weight, while also reaffirming the roll of carbonate chemistry, both during growth and in the depositional environment. This paper represents an important contribution to understanding the mechanisms of and interpreting differences in foraminiferal weight in the fossil record. Overall the paper is well structured and well written. I have highlighted below a few areas where the authors make some broad assumptions in their reasoning, which if addressed directly, could further strengthen this manuscript.
"Henehan et al. have, in their discussion, put forward some interesting ideas about the mechanisms underlying observed trends in calcification intensity in different sized G. ruber. *However, the extrapolation of this to all foraminifera (small/large, planktonic/benthic, juvenile/adult) is in my opinion overreach. This line of reasoning seems to equate adult foraminifera from small species with earlier ontological stages in larger species. However, it is unlikely that size alone is a meaningful determinant of physiology and calcification mechanisms across such a diverse group of foraminifera and ontological stages. I would recommend that the authors either remove these sections on pages 9-10 (and Fig. 5) or rework this discussion to better support and address these assumptions."*

Response: In light of the concerns of the reviewer, and the similarity to those concerns also expressed by reviewer 1, we have reworked this section of the manuscript. Specifically, we have:

- Removed the benthic foraminifera from Fig. 5 so as to reduce the emphasis on commonality of benthic and planktonic foraminiferal biomineralisation behaviour.
- Restructured the discussion in this section to more clearly separate hypothesis from subsequent treatment of empirical support.
- Explicitly stated the distinction between juvenile individuals and adult individuals of small species, and highlighted that at this time it is unclear to what extent these

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two groups may be considered analogous in terms of calcification behaviour.

Comment: *"The novel approach presented in the methods for quantifying calcification intensity in cultured foraminifera could be widely used, but raises some questions. This metric relies on the assumption that foraminifera of a single species from a certain locale will have a consistent size/mass relationship, such that an initial mass can be predicted from size. However, the authors show that environmental conditions (carbonate chemistry) can significantly alter the size/mass relationship. This would seem to contradict the underlying assumption of consistent initial size/mass. This apparently contradiction could be made explicit and addressed."*

Response: The reviewer is indeed correct that environmental conditions can likely alter the relationship between size and mass. It is true that at other locations the size-mass relationship we observe may not be valid, and so we add the explicit recommendation that the relationship between size and mass be verified and/or recalibrated at new culture locations before attempting to use this metric (Section 2.3, Page 5, Lines 5-7).

Comment: *"For example: Was anything done to constrain the environmental conditions of the foraminifera used to establish an initial size/mass relationship? How do the conditions at collection of these samples compare to those at the collection of cultured foraminifera?"*

Response: Our non-cultured samples used to devise a size/mass relationship were taken from numerous tows from the Gulf of Eilat over the course of several years, and with each tow open ocean Eilat seawater was sampled for pH measurement at or close to the site of towing. Despite temporal variability, the tows fall within a narrow range of ocean pH 8.10 $\pm$ 0.05 (2se). This is close to the midpoint of the pH range of

our culture experiments. What's more, these individuals were pooled from the same tows that yielded the individuals that went into culture, and so there should not be any significant difference between the conditions at collections for culture vs. those in the size-mass calibration. Therefore, the findings from our culture experiments are robust. We now make these points in the manuscript Section 2.3, Page 5, Lines 2-7).

Comment: *"The R^2 of the initial relationship is also not very high (0.61), suggesting quite a lot of variability in individual foraminifera size/mass - could Henehan et al. give an indication of the uncertainty this would introduce into the calculation of calcification intensity in a cultured foraminifera?"*

Response: The reviewer is correct in the assertion that there is some scatter around our open-ocean size-mass relationship. However we stress that much of this scatter is likely to have arisen from measurement error, rather than true physiological variability. In particular, instrumental uncertainty on microbalance measurements is large in proportion to absolute shell mass. However, the absolute measurement uncertainty is independent of either variable, and the sample number is so large, the regression line itself is likely robust. Importantly also, by definition, our regression relationship is structured so that variability in the tow data-points is normally distributed around our line. Therefore there should be no systematic bias introduced into our culture CI data.

We do recognise that the scatter in the prediction intervals, if propagated through each individual tests' CI measurements, would produce a sizeable range in individual tests' CI- particularly for those foraminifera that did not add much mass in culture. However, for a number of reasons we suggest this shouldn't detract from the main findings of the paper. Firstly, the regression line is applied equally to all individuals and all experimental pH treatments, and so given that the error in the regression should be non-systematic, the foraminifera used in the size-mass relationship were towed at the

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Discussion paper

same time as culture specimens, and the water they were towed from was towards the midpoint of our culture pH treatments, relative changes between experiments should be robust. Secondly, each pH experiment consists of a combination of several individual CI datapoints within an experimental treatment, and so provided the sample size is large enough, the error on each individual test's CI calculation introduced from the size-mass calibration is averaged out on the treatment level. Since we recognised that the sample size within each treatment has a large effect on the uncertainty of each treatment (given the regression error and inter-individual variability) we assign error bars on our culture experiments based on sample size. We calculated this uncertainty by repeatedly subsampling smaller sets of individual foraminifera from one of our larger experiments with > 100 individuals, and noting the deviation of the mean of each subset from the true mean value (see attached Figure). Therefore the bounds of uncertainty given in the paper do incorporate the uncertainty stemming from interindividual variability and scatter in the size mass calibration.
The models that we built to simulate CI change through ontogeny are also grounded with this same size-mass relationship. Because there is some considerable scatter around this relationship, as the reviewer states, we allowed our models to vary within a root mean sq. error (RMSE) of 3.12 around this observed relationship. This permitted variability in modelled size weight relationship of up to approximately twice that seen in our sampled natural population. Therefore the conclusions drawn from our model relationships stand even when considering the large residual scatter in the tow measurements.

Comment: *"Minor: The authors show that size-dependent calcification intensity is responsive to carbonate chemistry. Given this, they may wish to add an acknowledgment or brief discussion of the existing literature on how various environmental factors, like temperature, can impact shell size and growth rate (e.g. Schmidt et al., 2004 or Lombard et al., 2010)."*

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Response: We have now acknowledged this on Page 13, lines 6-8.

Comment: *"Line 14: edit "change changes"*

Response: Change changes changed.

Figure Caption: The relationship used to calculate our bounds of uncertainty. With repeated subsamples from a population of cultured individuals, the deviation of the mean of that subsample from the true mean can be calculated. This allow us to consider the effect sampling small numbers from a population with a large degree of inter-individual variability.

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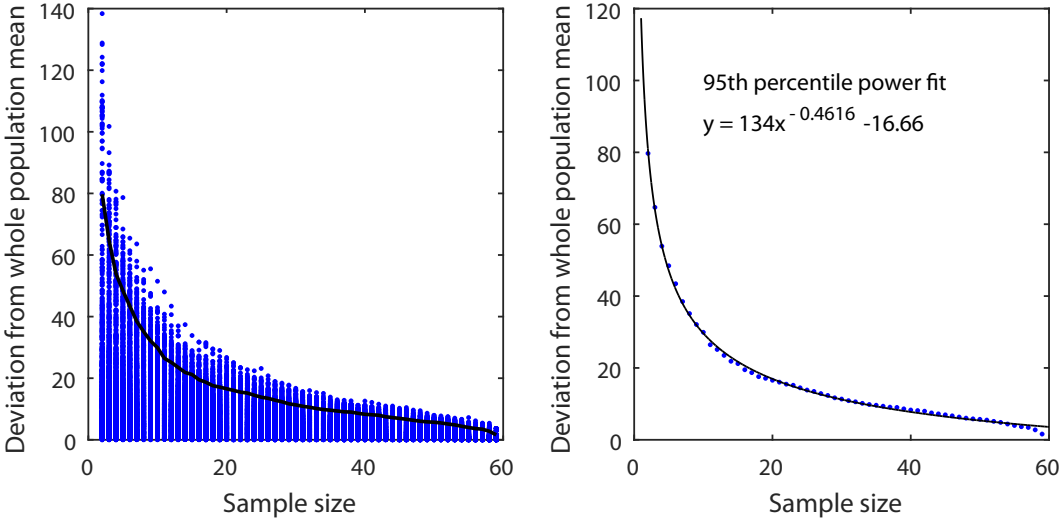

Fig. 1. Calculation of uncertainty from sample size.

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