

Interactive comment on “Effects of ocean acidification on calcification of symbiont-bearing reef foraminifers” by K. Fujita et al.

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We are pleased to receive two constructive reviews concerning our manuscript entitled “Effects of ocean acidification on calcification of symbiont-bearing reef foraminifers”. In response to the referees’ suggestions and recommendations, we have revised the manuscript as follows.

Referee #1 (Dr. J. Hohenegger)

Comment: It experimentally strengthens the assumption, that hyaline larger foraminifera positively react to increased acidification as induced by global warming than the porcelanous larger foraminifera.

Reply: We acknowledge the referee’s important suggestion. The implication of this
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culture experiment for the geologic record of large benthic foraminifers was discussed in the Discussion section.

Referee #2 (Dr. L. J. de Nooijer)

Comment #1. Throughout the manuscript, the authors refer to the three species by their genus-names. I recommend adopting the standard annotation (i.e. *B. sphaerulata*, *C. gaudichaudii* and *A. hemprichii*).

Reply: We changed the genus name to the species name throughout the text.

Comment #2. Since this is the first paper in which their culturing set-up is described, I suggest that a schematic drawing is included that shows the relation between towers, gas mixers, culture vessels, water bath, lights, etc.

Reply: The schematic drawing of our culturing set-up was added as a new figure (Fig. 1).

Comment #3. The data may be presented a bit more condensed. The difference between the two clone populations is generally low and therefore the two figures from one species may better be combined somehow. Where is the dotted line in clone population of *Calcarina* (Fig 2, upper panel)?

Reply: We consider that one graph including two population data appears to be complicated. We also consider it important to clearly show the variability in growth data on each clone population, in response to the referee’s comment #5 and summary comments. Therefore, we decided not to follow this recommendation, and remained the related figures as is. In Fig. 2A, a dashed line was overlapped with a solid line. We changed the two lines to short arrows in this graph as well as other similar graphs, in order to clearly show the shell weight and diameter of initial individuals.

Comment #4. Could the authors assess whether the foraminifera grew throughout the experiment? Since the weight and size were only determined after 12 weeks, it may be that eventual growth rates are underestimated (i.e. when all growth occurred in the first

weeks). Because future culture studies may use the data presented here to compare to, I recommend stressing the uncertainties in the estimated growth/calcification rates.

Reply: We agree with the referee that growth rates are averaged or underestimated. We did not conduct time-course measurements during this experiment to avoid the loss of cultured specimens and to minimize stress to them during manipulation. However, our previous results on *Marginopora kudakajimensis* (Table 1 in Kuroyanagi et al., 2009) statistically confirmed that shell size increased with time over the range of seawater pH examined (from pH 8.3 to 7.7; NBS scale). On the other hand, culturing studies of other species of reef foraminifers (*Amphistegina* spp.) showed that growth rates of juvenile clones were faster than those of matured ones (Hallock et al., 1986). Thus, the uncertainties in the estimated growth (calcification) rates were mentioned in the section 2.5 Measurements.

Comment #5. Looking at all the results together, there seems no clear response of the cultured foraminifers to the supplied pCO₂'s. It may be that the introduction of altered seawater carbonate chemistry caused stress (particularly at the beginning of the experiment) and thus impacted determined growth rates. On the other hand, Langer et al. (2009. *Biogeosciences* 6: 2637) have shown that different strains (subspecies) of coccolithophores may respond differently to induced ocean acidification. If such results are valid for foraminifera too, the difference between the clone populations may thus be (partly) explained. These alternative explanations for the observed responses have to be included in the manuscript.

Reply: In response to these comments and the referee's summary comments below, a new subsection was added in the Discussion to discuss variability in the data, possible problems associated with culturing experiments, and factors other than carbonate chemistry that would impact calcification rates.

Regarding the first comment that the introduction of altered seawater carbonate chemistry caused stress particularly at the beginning of the experiment, reef foraminifers are

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usually subject to a large diurnal variation in pCO₂, which ranges from 100 to 1000 ppm (e.g., Suzuki, 1994). Thus we consider that our studied ranges of carbonate chemistry are not beyond the tolerance limit of the physiology and growths of reef foraminifers. However, cultured individuals were not pre-incubated in altered pCO₂ conditions prior to experiments. Thus, the introduction to altered seawater carbonate chemistry may have required time for foraminifers to acclimate and therefore caused them stressed particularly at the beginning of the experiment.

Although growth rates of cultured individuals were similar to or lower than those of field populations in the same subtropical locations, relatively low growth rates are considered to be caused by environmental variables other than carbonate chemistry (e.g., light, temperature, water motion, and food availability), which might have not been optimum conditions for the studied foraminifers. Nevertheless, since cultured individuals were maintained in the homogeneous conditions except for pCO₂, observed differences in the growth rates can be interpreted to be caused by different pCO₂ levels.

Regarding the second comment, the possibility of different responses of phenotypes in reef foraminifers to elevated pCO₂ was discussed to explain the variability in growth data between clone populations.

Comment #6. The introduced ocean acidification has also altered the [DIC]. Could increased DIC concentrations have had a positive effect on the growth rates? Please include these values in Table 1.

Reply: The [DIC] values were added in Table 1. Effects of increased DIC concentrations on foraminiferal growth rates were mentioned in the Discussion section.

Comment #7. The discussion about the possible difference in utilization of inorganic carbon species is highly speculative. Modifications of the internal (and external) pH by foraminifers show that the ratio between dissolved carbon dioxide/bicarbonate/carbonate is easily modified. Therefore, the supposed use of bicarbonate vs carbonate between species should be omitted.

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Reply: Sentences including speculations on the carbon species used for calcification were deleted. Our speculations were based on inorganic carbon uptake studies using ^{14}C tracer techniques by ter Kuile et al. (1989). We only cited the main results of their experiments, which were related to our experiments. We also mentioned the possibility that the ratio between dissolved carbon dioxide/bicarbonate/carbonate can be modified by foraminiferal protoplasm.

Comment #8. Could it be that production of new chambers (i.e. calcification) only takes place as the foraminifer's cells grow? In that case, the inorganic carbon availability and pH may have a small effect on calcification compared to cell growth. . . This possibility should be mentioned.

Reply: For *Baculogypsina sphaerulata* and *Calcarina gaudichaudii* (hyaline species), we agree with this comment that inorganic carbonate availability and pH may have a small effect on calcification compared to cell growth. Enhanced photosynthesis by algal symbionts at intermediate pCO_2 levels may promote foraminiferal protoplasmic growth, which in turn possibly stimulates chamber formation (i.e. photosynthetic fertilization effects). In addition, photosynthates (carbohydrates) may be used for the organic matrix in a foraminiferal shell, which is a possible link between photosynthesis and calcification (Hallock, 1999). On the other hand, for *Amphisorus hemprichii* (porcelaneous species), seawater carbonate chemistry seems to largely influence the calcification, compared with photosynthetic fertilization effects. These possibilities were discussed in the Discussion section.

Comment #9. Were there any observed differences in the appearance of the foraminifers between the different conditions? Are there SEM pictures available? Could the authors extend their results by estimating chamber wall thicknesses (are the results presented here somehow comparable to the inferred relation between OA and planktic chamber wall thickness suggested by Moy et al. (2009. *Nature Geosciences* 2: 276), de Moel et al. (2009. *Biogeosciences* 6: 1917) and Barker and Elderfield (2002. *Science* 297: 833))?

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Reply: We added SEM pictures of average-sized cultured specimens from each of different pCO_2 conditions (as a new Figure 2). Comparisons of the SEM images indicate that differences among treatments were mostly due to different growth rates (i.e. the number of chambers added during the experimental period). However, we do not deny variability in shell wall thickness among different pCO_2 levels, because our previous paper (Kuroyanagi et al., 2009) showed the shell weight of *Marginopora kudakajimensis* decreased with lowering seawater pH for specimens with an identical shell diameter. This possibility needs more detailed investigations and will be discussed in a separate paper. These were addressed in the Discussion section.

Comment #10. What do the results imply for the use of large benthic foraminifers as "first indicators" in reef ecology as OA continues (as mentioned in the Introduction)?

Reply: Our findings suggest that ongoing ocean acidification might favor symbiont-bearing reef foraminifers with hyaline shells at intermediate pCO_2 levels (580 to 770 μatm) but be unfavorable to those with either hyaline or porcelaneous shells at higher pCO_2 levels. Thus, we propose comparisons of growth rates of reef foraminifers with previous rates as indicators of the degree to which ocean acidification will be proceeding. This was mentioned in the Discussion.

Summary comment: In summary, I think the Discussion should be less speculative, can be condensed considerably and instead should focus more on 1) variability in the data as such, 2) possible problems associated with culturing studies and 3) other factors than pH that may impact calcification rates.

Reply: As already explained in the reply to the related comments above, the Discussion section was revised throughout following these comments.

We should like to thank the referees for their helpful comments, which greatly improved our manuscript.

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