**McIntyre-Wressnig et al., Non-lethal effects of ocean acidification…**

This paper describes a study in which foraminifera were cultured at high $pCO_2$ levels. The authors studied survival of the foraminifera, fitness and test microfabric. The material is appropriate to Biogeosciences but I do not recommend publication of the paper. This is for two principal reasons. I think that the data set is too small to allow any significant effects in foraminifera performance to be determined. I also think that the carbonate system has not been adequately characterized in the experiments.

**Main comments:**

1. The dataset for this paper is very small. In the abstract the authors state that they have maintained two different foraminifera species at ambient and two elevated concentrations of $CO_2$ (1000ppmv and 2000ppvm). It appears that the experiments were conducted in two sets: first the controls and 1000ppmv exposures followed by a second series of controls and 2000ppmv exposures. On reading the paper it becomes clear that in one species, *A.angulatus*, the foraminifera were overgrown by algae in the first series of experiments (control and 1000 ppmv). In the second series of experiments (controls and 2000 ppmv), survival of this species in some plates (the exposure vessels) was very poor (4% in the controls foraminifera after 1 and 6 weeks). These are the control foraminifera and the poor survival rate reflects issues with the experimental design e.g. light availability, seawater chemistry in the plates etc. I do not think that the authors can hope to elucidate accurately the effects of elevated $CO_2$ on this foraminiferal species until they are able to maintain successfully most of the control individuals over the duration of the experiment. These data should be removed from the paper.

This leaves the second foraminifera species, A. gibbosa. For each experiment the authors have examined 24 individual foraminifera. This seems to be a very small number of individuals and I wonder if the number is too small to be representative of the population. In particular, the 24 individuals are maintained within a single plate which is fixed in one location in the culture incubator. Variations in light, temperature etc between plates may affect the results of the study, as noted by the authors (p9177, line 20). Survival rates for this species in the controls range from ~60% (6 weeks at 2000 ppmv) to ~100% (1 week at 2000ppmv). Because the authors have exposed 24 individuals within a single plate in each treatment there are no estimates of error for survival in each treatment. Given this wide range in survival rate in the control individuals it is perhaps not surprising that the authors do not discern an effect of $pCO_2$ on survival. The authors conclude that fitness and survival is not affected by $pCO_2$. This could be an artifact of the very small sample sizes (and large error bars associated with the ATP analysis and no error bars associated with survival). It is not clear that they have they tested this statistically. It would be better to repeat the experiment using a much larger number of individuals which are distributed between several plates. This would enable the authors to calculate an error for the estimate of survival which encompassed experimental variations.

2. Carbonate system parameters - I do not think that the carbonate system has been adequately characterized in these experiments. To assess total alkalinity and DIC the authors have sampled seawater, kept separately from the foraminifera in the incubators. The authors state that they sampled seawater from the incubation cabinets in weeks 1, 2, 4 and 6 (P9173, line 7). But they do not report this data as they have concerns that the increases in salinity in the seawater vials over the experiment have affected their measurements. So their estimates of the carbonate system come from the week 1

measurement only. They use estimates of uncertainty from another experiment (conducted at 7ºC instead of the 25ºC used in the current paper) to infer the changes in the carbonate system in the seawater in their experiments. I do not think that this is appropriate. Multiple measurements throughout the duration of the experiment are required to demonstrate the stability of the system during the current experiment. Without these data it is not possible to be confident of the incubator conditions.

Furthermore it is not clear that the sampled seawater reflects the seawater chemistry in the seawater in which the foraminifera are maintained. Each foram is maintained in 1.7 mls seawater within each well of the plate. This is a small volume and it seems likely that photosynthesis (by the algal symbionts), respiration (by the symbionts and by the foram) and calcification (by the foraminifera) will all affect the carbonate system in the seawater surrounding each foraminifera. Variations in the chemistry of these small volumes of water are likely to be much larger than any variations observed in the isolated seawater samples maintained in the incubator. The authors need to characterise the chemistry of the seawater in which the foraminifera are maintained. If the authors have estimated the amount of calcification of each individual foram, then they can calculate how this affects the alkalinity of the seawater. It should even be possible to sample 1 ml of seawater from each well at the end of the experiment to assess changes in alkalinity. The authors could use measurements of respiration etc. to calculate how this affects seawater chemistry.

**Other points:**

P9166, line 11 and at other places. Remove '(A.)'.

Line 19 typo - soluble

P9167, line 2 typo - led

Experiment overview - I found the description of the experiment somewhat confused. Rewrite the methods section to make the methods clearer. State if the 1000ppmv and 2000ppmv experiments were conducted at different times. When were they conducted? Were foraminifera collected from the field at different times? How could a staggering of experiments affect the study results? Explain how the foraminifera were housed in each experiment. What is the rationale for the rebound treatment? Why were only some foraminifera photographed at the start of the experiment? What were they used for?

P9172, line 1 What is the error (±1.8)? Is it 1 standard deviation?

Line 14 typo - 'to' should read 'in'

Section 2.5 Presumably these estimates of error in alkalinity and DIC are from the other experiment conducted at 7 ºC. Estimate how much the salinity in the plates varied (by knowing how much distilled water you had to add to each well.

Section 2.6 Explain the time dependent nature of the ATP response and explain why you did not photograph all the forams.

Section 2.8 'None of the A. angulatus specimens… precipitated CaCO3'. How do you know this? Did you compare photographs of each individual at the start and end of the experiment? Is it possible that forams did not accrete new chambers but that they deposited calcite over the surface of the existing test?

Section 3.2 I do not think you can make any meaningful observations about survivorship in A. angulatus if survivorship in the controls is only 4%.

Section 3.3. Explain explicitly how you have estimated growth in each foraminifer. Do you mean direct or rebound individuals here? If you are assessing the effects of elevated $CO_2$ then you should use the direct individuals (otherwise you may be measuring calcification in the rebound phase), but you state that you photographed only 6 specimens from the direct plates. This is likely to be too small a number of individuals to be representative. What are the errors here (9.2%), 1 standard deviation or what?

Section 3.5. Are you imaging the fabric of chambers deposited during the experiment or the fabric of chambers which had already been deposited before the experiment started? It is not clear in this section if you are referring to control or treated individuals. Line 19 - it does not appear that the entire test surface has dissolved in the images shown in figure 5. Line 15 typo 'than' should read 'from'.

Discussion. How quickly does ATP react to changes in environmental conditions - within hours days etc? How have you tested that reproductive yield is not affected by $CO_2$?

Nicky Allison, University of St. Andrews UK, September 2011