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## ***Interactive comment on “Non-lethal effects of ocean acidification on two symbiont-bearing benthic foraminiferal species” by A. McIntyre-Wressnig et al.***

### **Anonymous Referee #2**

Received and published: 26 September 2011

Review of the manuscript entitled “Non-lethal effects of ocean acidification on two symbiont-bearing benthic foraminiferal species” by McIntyre-Wressnig, Bernhard, McCorkle, and Hallock.

General comments: The present manuscript investigates the effect of acidified conditions on the biology (survival, loss of symbionts, reproduction) and calcification (from surface electronic microscopy) on two species of benthic foraminifers. This is an important topic knowing the present and future perspective of ocean acidification. This manuscript is well written and by contrast to several publications, does not address only the effect of ocean acidification on calcification. The main findings are that acidification

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does not impact the survival rates of foraminifers but that, loss of symbionts, reduced growth and dissolved areas were observed in acidic conditions. These findings are then interesting because they raise new consequences of ocean acidification.

However, some important methodological points need strong clarifications, notably how salinity was controlled (or in what extend it was not controlled) and more importantly, the way the survival rate was assessed seems to me inaccurate (except if the way it is described is a mistake). Effectively, the authors used an ATP threshold to determine if foraminifers are alive or not originating from Bernhard & Reimers (1991) originating from a deep-sea foraminifer (page 9175). In order to adapt this threshold on their foraminifer species, the authors had measured wet (full of water) and dry weight of their shells and thus got to the conclusion that "The mean difference between dry weight and wet weight was 20 % for both species and was considered to be the contribution of cytoplasm to the overall volume.". This assumption is wrong and only valid if calcium carbonate have the same density as water. . . which is not the case ( $d=2.71\text{g/cm}^3$ ). If I take this assumption (shell = 80% volume) and a  $1\text{mm}^3$  shell, then the cytoplasm (or water) should only contribute to 8.4% of the wet weight (Wet-dry/wet). . . which is far from 20%. On the contrary if I take the assumption that water weight is 20% of the total wet weight of a  $1\text{mm}^3$  shell, this means that water fill 40% of volume (which is twice as the presented volume). Thus if this mistake impacts proportionally the chosen threshold, the "survival" threshold should be multiplied by two. . . . And thus all the "survival" results should be calculated again. I suggest below two calculation methods for identifying for each shell the volume occupied by water.

Method 1: weight of water (because of density =  $1\text{ g/mm}^3$ ) can directly be converted in volume and thus compared to the volume of the shell (if measured; if not. . . take method 2).

Method2: WW= wet weight; DW= dry weight; W1= weight of water (only); V1=volume of water;  $d_1$ =density of water; V2=volume of calcite;  $d_2$ =density of calcite. Volume of calcite (V2) can be calculated from weight such as  $V_2=DW/d_2$ ; V1 can be calcu-

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lated from  $V1 = (WW - DW) / d1 \dots$  and thus the ration between V1 (or V2) to total volume ( $V1 + V2$ ) can be calculated.

Thus because of this method problem, it is hard to judge whether or not the depicted results are correct, and thus the present manuscript needs either strong clarification of this method, or strong corrections in the methods which would imply changes in results and conclusions. Consequently it is certainly premature to judge whether or not the current manuscript deserves to be published in Biogeosciences.

Detailed comments:

Page 9166 Line 3 (and everywhere in the manuscript): please replace “microfabric” by “microstructure”

Page 9169 Line 26: “palm-sized” replace by hand or give a size

Page 9170: please provide in situ temperature and salinity/ please provide also the salinity in the experiments... those could be different and thus may have induced a stress of the animals.

Line 7-8: transport to WHOI

Line 15: “organism”

Page 9171 Line 6: precise what are the two (“each”) experiments, explain also that the two experiments were proceeded at different times (explaining then the confounding double controls which are not the same) Line 12-13: “Only the Control and Direct treatments were used for test microstructure analysis. “ Why? Line 13: please provide the volume of water per well. One foram is placed in each well?

Page 9172, Line 22-26: do you not think that this “below optimal light” is not responsible for the loss of symbionts and other observed impact of 2000ppmv treatment... please discuss

Page 9173 Line 5: in 22ml you will not have the same evaporation than in the wells...

**BGD**

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this (and potential consequences) needs to be discussed Line 9= please provide the concentration of HgCl<sub>2</sub> Line 28: You will not have the same evaporation in these conditions (7°C) than during the experiments (25°C).

Page 9174: how much individuals were used for survival?

Page 9175 Line 17: please provide the variability

Page 9176, Line 5-6: how “normal” and “changed” microstructure were identified?

Page 9177 Line 14-16: I did not understand: more than 90% died after 1 weeks... but only less than 60% died after 6 weeks... Did all these survivors rates are calculated relative to time=0? If yes, what could explain such a discrepancy? Line 24-25; what parameter you used for determining the growth? 55% increase in size? Number? Weight? volume? (this is also not indicated in material and methods)

Page 9178 Line 26-27: please provide an example of control to judge the difference “at 6 weeks” rather than “after 6 weeks” (they had born during the experiment)

Page 9179 Line 27-28: Speculative

Page 9181 Line 10-12: those two propositions are opposite... then you did not know if they are stressed or not (they reproduce in both cases)

Table 2: Please provide full explanation of “SEM”

Figure 2: I did not understand : “control” is supposed to be at ambient pCO<sub>2</sub>. Then why control are different between 1000 and 2000ppmv. Did the two concentrations have been done at different times? If yes precise it clearly in the material and methods. The difference between the two controls (ambient pCO<sub>2</sub>, but different times?) are higher than between the conditions tested (especially for ATP content)...

Fig 4: Please explain what are the difference between 1) 2) 3) and 4)

Fig 7: comparison with control?

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