

Interactive comment on “Experimental evidence for foraminiferal calcification under anoxia” by M. P. Nardelli et al.

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Dear Editor Prof. Kitazato,

We thank both reviewers for their constructive comments and mainly positive feedback. We here address only the major points; we have followed most of their suggestions regarding grammar and technical details and feel no need to discuss those further.

Kind regards,

Maria Pia Nardelli and co-authors

Answer to anonymous referee #1

Comment: “Please add quantitative data to the abstract”

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Reply: As suggested by the referee we added quantitative data on calcification rates of each species in the abstract.

Comment: “The percentages are really quite low. That, when taken with the small populations, makes the argument not very convincing.”

Reply: We are aware that the numbers are not very high, and we state it in the text (i.e. line 5 pag. 4677). However the fact that few individuals did calcified under anoxia during the experiment is important as it demonstrates they can do it.

Comment: “For experiment 1 (*Ammonia tepida*), 1-4% added calcite (n= 50 per treatment). How can there be 1% of n=50? If one specimen calcified, it would be at least 2%.”

Reply: The given percentages are averaged over the three replicates (n=50 each). In supplementary materials real numbers for replicate and averages are given. For *A. tepida* the average of specimens that calcified new chambers varied between 0.78 and 4.07 %, rounded off in the manuscript to 1-4%.

Comment: “For *B. marginata*, about 2.5 specimens per treatment calcified under anoxia: 2.3-3.3 cm, total seeded population = 31 or 32; 8% (+/- 4%) calcified = 1-4 specimens. Not impressive numbers.”

Reply: Please see answer to question 2.

Comment: “Were the foraminifera labeled with calcein in an aerated habitat? Were they allowed to acclimatize prior to placement in the hypoxic or anoxic core sections? The authors are urged to explain how acclimatization, or lack of acclimatization, impacts their results.”

Reply: The foraminifera were labeled in aerated conditions, in plastic jars filled with few millimeters of sediment and air bubbled artificial sea water (+ calcein). They were introduced into the experiment without prior acclimatization. The lack of acclimatization however does not seem to have impacted foraminiferal health as survival rates in anoxic

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layers are not significantly lower than in the oxic layers, at least for *B. marginata* and *A. tepida*. Also for the species *C. laevigata* that showed a higher sensitivity to anoxia, the calcification of some of specimens in the anoxic layer suggests that their death under anoxia was not immediate (time needed for calcification), therefore suggesting that it could not be related to lack of acclimatization.

Comment: “It is reassuring that the authors included the significant caveat on the issue of the sediment homogenization near the end of the manuscript. But, in addition to this issue, there is the issue that the segmented cores used for the foraminifera experiments may not exclude oxygen as the authors assert.”

Reply: The lack of oxygen penetration between the core layers was controlled in different ways. Oxygen profiles were measured in the segmented cores during the first 30 days of experiment, before the introduction of foraminifera. No oxygen was detected below the 3 mm oxygenated superficial layer. Moreover, after the introduction of foraminifera, the segmented cores were covered with plastic bags filled with sediment, in order to further avoid any possibility of oxygen penetration among the layers. The sediment of each layer was observed at the end of the experiment to check for color changes due to the presence of oxygen. The anoxic layers showed light grey to dark grey homogeneous color (see figure 1 below). No light sediment associated to lateral oxygen penetration was observed at sub-superficial layers.

Comment: “The presented geochemical profiles were obtained from unsegmented cores, not those that housed the foraminifera. Additionally, the presence of foraminifera could have affected the geochemistry of the sediments. Thus, the geochemical profiles are basically worthless and serve no point of comparison. Therefore the results are tenuous at best.”

Reply: All the cores were filled with homogenized sediment. This was confirmed by the fact that the oxygen profiles obtained for segmented cores at the start of the experiment were completely comparable to that measured on the geochemical cores

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(same oxygen penetration depth). For this reason we assume that the profiles obtained on geochemical cores during the whole experimental time were representative of foraminiferal cores too. The experimental needs for foraminiferal analysis and geochemical analyses on pore waters prevent the possibility to perform b on the same sediment cores. For this reason other similar studies, where foraminiferal and geochemical analyses were needed, used the same methodology, performing both the analyses on different cores, assuming that they are enough homogenous to be considered equivalent. See for example the study by Koho et al. 2011, FEMS. Concerning the contribution of foraminifera to the geochemistry of interstitial waters, we estimated that it was not significant based on the estimation off their contribution to oxygen fluxes. In fact, oxygen is the most sensitive geochemical parameters, dependent of organic matter contents, and influencing the production of all others geochemical products of early diagenesis. We estimated the contribution of foraminifera to the oxygen flux of the experiment 1 (with *Ammonia tepida*) which showed the most important oxygen flux (=lowest oxygen penetration depth). On the basis of the measured oxygen profiles and porosity of sediment we estimated a flux of 7736 mmol O₂ cm⁻² h⁻¹ for the initial time of the experiment. Using the respiration rates of 2.01 nl O₂ cell⁻¹ h⁻¹ measured for *A. tepida* by Geslin et al. (2011) and considering the number of specimens added to the oxygenated layer (n=50), we can estimate a total oxygen respiration of 5.7E-06 mmol 50 cell⁻¹ h⁻¹. If we compare this value to the oxygen flux for sediment core (core area= 12.6 cm²), we obtain that the contribution of added foraminifera to the oxygen flux corresponding to 5.86E-09%, which is not significant. Hence, we are confident that the profiles obtained on the “geochemical” cores can be considered representative of “foraminiferal” cores.

Comment: “For the *A. tepida* experiment, what is meant by “filled with N₂-flushed ASW”? The text notes that the subsurface intervals were loaded with 50 *A. tepida* and then filled with seawater. These are supposed to be down core samples. If seawater was added, then each interval was by definition, a sediment/water interface. This along with the possibility of non-air-tight cores throws significant doubt on the results about

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calcification or even survival under anoxia.”

Reply: To introduce foraminifera in the cores we needed to pick them with a Pasteur pipette. It was therefore inevitable to introduce a small volume of water in the core system. To avoid re-oxygenation of anoxic sediment layers the water used for foraminiferal transfer from eppendorf tubes to sediment cores was briefly flushed with N₂ in order to remove oxygen. The quantity of introduced water was very limited (0.5 ml maximum) and it was rapidly absorbed by the sediment. Therefore there was not a water/sediment interface at each layer. However the sentence was modified for a better comprehension (lines 108-110).

Comment: “The basis for the statement “that during short anoxic periods, foraminifera will continue to calcify, at least at the sediment-water interface” is not clear. What short interval? 60 days is a short interval?”

Reply: We agree with referee #1. We specified that we talk of a period of 60 days (line 337).

Comment: “What is the basis for saying in the first sentence that “oxygen depletion is one of the most severe environmental stressors in marine ecosystems”? There is an entire ecosystem that exists in oxygen depleted marine habitats; these organisms are not stressed by such conditions.”

Reply: The sentence is not only about foraminifera. A strong decrease of all marine faunas after sporadic anoxic events, especially in coastal areas, is reported by hundreds of publications (e.g. Pörtlner et al., 2014 IPCC report WGII AR5 Chapter 6).

Comment: “The statement that fossilizing calcareous tests of foraminifera “register the geochemistry of seawater” is a major overstatement. In some cases we think they register the seawater in which the foraminifer grew, but in some cases they do not. Please be sure to be accurate in assertions. Using the word “proved” in scientific literature is an unwise choice.”

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Reply: We agree with referee #1 and we changed the text after the suggestions (lines 32 and 38).

Comment: “The statement about “recent discovery of facultative anaerobic metabolism” in foraminifera and citing one paper from 2006 is misleading. There are a number of earlier publications that showed facultative anaerobic survival of foraminifera. The authors are urged to cite these papers.”

Reply: Even if several other anaerobic metabolisms have been proposed to explain foraminiferal survival under anoxic conditions (e.g. Bernhard and Bowser 2008, Bernhard and Alve 1996, etc.) at present day the only directly observed and quantified anaerobic metabolism performed by foraminifera is denitrification. However we added citations of earlier proposed anaerobic metabolisms to the ms (lines 40-41). We also deleted the adjective “recent” referred to the discovery of denitrification.

Comment: “How was sediment sieved over such a fine screen without added liquid? Did the authors use a 38 micron screen? The text notes a <38 micron sieve.”

Reply: The sediment was sieved on a 38 μm mesh sized sieve, using a brush to press the sediment through the sieve. We added this detail to the materials and methods paragraph (line 56).

Comment: “How do the authors know that the FDA signal they see is foraminiferal and not bacterial?”

Reply: Contrary to the foraminiferal cell signal, FDA signal given by bacterial activity is spotty and not homogeneous in the whole foraminiferal test. The difference between the two signals is easily discernable.

Comment: “France is not in the West Atlantic, per the statement in Section 2.2.”

Reply: West was referred to French coast and not to Atlantic ocean. The text was modified for unambiguity.

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Comment: “How did the authors minimize evaporation during the 60 day experiment?”

Reply: The aquaria were covered with a non-hermetical cover to reduce evaporation. Weak salinity variations (daily measured) due to evaporation were corrected by the addition of MilliQ water.

Comment: “How might the lack of feeding during the experiment impact the results?”

Reply: We think that the major possible impact of the lack of fresh organic matter could be lower than expected survival (and possibly calcification) rates, as already suggested for *A. tepida* experiment (lines 244-246). However the choice to avoid fresh organic matter inputs on the top cores were motivated by the priority, for the experimental purposes, to have geochemical pore water conditions as stable as possible, to better identify the geochemical conditions of each layer where foraminifera could survive and calcify. We clarified these aspects in paragraph 2.1 (lines 59-61).

Comment: “The entire section on pH profiles is poorly written, awkward, and undecipherable.”

Reply: We agree with the referee. The entire section has been rephrased.

Comment: “Why are “nitrates” plural?”

Reply: It is the sum of nitrate and nitrite. We better specified it in paragraph 2.4.3 (line 157).

Comment: “The authors are reminded that anoxia by definition is a lack of oxygen, thus “completely anoxic” is poor word choice.”

Reply: We agree and we deleted “completely” throughout the text.

Comment: “The sentence on page 4677 lines 7-9 belongs in the Discussion, it is not a Result.”

Reply: We agree with the referee. We deleted the sentence, as the same concept was

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already repeated in the discussion section.

Comment: “The authors are urged to not make overstatements such as “severely adverse conditions”

and “highly adverse”. Again, that is judgment from an anthropocentric perspective. “
Reply: We deleted these overstatements as suggested by the referee.

Comment: “It must be made clear that the papers cited in the first sentence of section 4.3 are examples and not an exhaustive list. Thus, “e.g.,” must be included for both of these sets of citations.

Reply: We added “e.g.” as suggested by the referee.

Comment: “The inference about juveniles being more susceptible to anoxia is pure conjecture. Please include citations of other studies that show this for other taxa. There are no life stages in foraminifera (no molt stages, no larvae).”

Reply: We modified the text to specify that the possible higher sensitivity and inability to calcify of juveniles is a hypothesis, that, however, at present day we cannot exclude yet (lines 350).

Comment: “It is not “generally assumed that no foraminiferal tests are produced during anoxic periods”. What about the pore-size proxy with respect to oxygen concentration (Kuhnt et al. 2013 DSRI; 2014 JFR)? What about elemental ratios (e.g., Mn/Ca; Groeneveld & Filipsson 2013 BG)?”

Reply: While calcification under hypoxia is assumed to happen and is the base for the investigation of several oxygenation proxies, there are not studies that investigated geochemical proxies on foraminiferal tests supposed to be calcified under anoxia. To our knowledge, the papers cited by the referee#1, as many other existing on paleoxygenation proxies, concern hypoxic conditions, but not anoxic ones.

Comment: “The title is not particularly accurate of the content, given much of the

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manuscript reports survival rates.”

Reply: The manuscript report both survival and calcification. However the real news of the presented results is the ability for some specimens to calcify under anoxia, as the strong tolerance of many foraminiferal species to short term anoxia is already known from decades. For this reason we decided to keep the title as it was.

Comment: “The awkward English phrasing, incorrect spelling, inconsistent spelling, and poor grammar must be corrected.”

Reply: We thank the referee for the suggestion and we will ask for English editing service for the final revised paper.

Comment: “The samples were kept refrigerated until laboratory.” What does this mean?”

Reply: We sampled sediment containing living foraminifera and we transported the samples in cold-boxes until laboratory where they were placed into incubators to keep them at constant (the same of sampling site) temperature. We clarified it at line 121.

Comment: “This aspect will be further discussed in the next paragraph”. The next paragraph noted nothing about the topic.”

Reply: We moved the sentence above. “This aspect” was referred to the energy (ATP) needed for calcification and possible metabolic adaptations under anoxia. This topic is deepened in the next paragraph as announced by the cited sentence.

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Fig.1 (left) 2.3-3.3 cm deep layer of experiment 2 showing homogeneous light grey color; (right) 0.3-1.3 cm deep layer of experiment 2 showing brownish surface (low oxygen concentrations still present) and grey to dark grey bottom, suggesting that no lateral oxygen penetration occurred at the bottom layer.

Fig. 1.

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