

## ***Interactive comment on “Foraminiferal survival after long term experimentally induced anoxia” by D. Langlet et al.***

**D. Langlet et al.**

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Received and published: 18 July 2013

Dear editor, Dear referee, we hereby provide you with our detailed answers to your remarks on our manuscript. Our answers to your remarks can be found below or in the supplement.

#AR1: Title: In my view, the title would be better if it indicated that the experiment was conducted in situ (not in the lab). As the text indicates, this is one of the few experiments of its kind that was conducted under natural conditions. The title should reflect this.

Authors' response: we agree with the Anonymous referee 1 and the title will be changed to “Foraminiferal survival after long-term in situ experimentally induced

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anoxia”.

#AR1: Abstract: The abstract needs to include the water depth of the cores examined. Metatranscriptomic results should also be mentioned.

Authors' response: the water depth (24 m) will be added on page 9244 line 3. A sentence explaining the metatranscriptomic results will be added to the abstract.

#AR1: Methods: Water depth should also be mentioned here (not just <50 m).

Authors' response: the water depth (24 m) is presented in section 2.1 page 9249 line 20. The “< 50 m” page 9248 refers to the general depth of the Northern Adriatic Sea.

#AR1: Although the EAGU is described in an earlier publication, relevant aspects, such as the diameter of the EAGU footprint on the seafloor, should also be mentioned here.

Authors' response: the information that the EAGU is a cubic plexiglass chamber of 0.5m side length will be explained in section 2.1.

#AR1: Page 9252 I disagree with the use of two different techniques to examine the 63-125 micron assemblage compared to the >125 micron assemblage. It seems to me that the potential biases introduced by processing these assemblages differently and examining one wet and one dry are much greater than any “statistical problems” that might arise from splitting. The study is too elegant to mix and match processing and examination techniques. I would not advocate these use of separate techniques in future studies.

Authors' response: The CTG method was designed to be used in wet samples, so the technique we employed for the > 125  $\mu\text{m}$  is not problematic (Bernhard et al., 2006). Two aspects can potentially be problematic with the treatment applied for the 63-125 $\mu\text{m}$  fraction: 1) the density separation technique can lead to a loss of foraminifera, and 2) the drying of the sample can affect the CTG coloration. For the first aspect, the denser fraction was checked and no hard-shelled benthic foraminifera were found. For the second aspect, several tests of the CTG response to the sample treatment (drying)

were performed prior to the definitive analyses. The results show that despite the drying of the sample it was still possible to differentiate between fluorescent and non fluorescent organisms. Consequently it seems that both methods do not lead to a significant loss of foraminifera and both methods permit to accurately determine if the foraminifera are living or not. Although we agree with the reviewer that it would have been better to use the same sample treatment protocol for both fractions, for practical reasons (too time-consuming to study the 63-125  $\mu\text{m}$  fraction wet) this was simply not possible. We think that we can say with confidence that the methodological bias is very small, if present at all. Nonetheless, as the reviewer suggests in regard to future studies, we will attempt a more uniform methodology in the future.

#AR1: The introduction of brittle stars to the chambers should be included in the methods (not introduced in the discussion; page 9257). The placement of macrofauna into the chambers apparently was to provide a biological oxygen indicator, but yielded interesting results because of the addition of food to the foraminiferal populations. However, the activities of oxygen- and food-starved brittle stars in an enclosed chamber likely created unnatural bioturbation of the sediments and may have altered vertical distribution patterns and densities in the initial 1 to 2 weeks.

The introduction of brittle stars as bio-indicators of oxygenation will be included in section 2.1 as suggested by the anonymous referee. We will elaborate a bit better that the organic matter input is not only due to the introduced brittle stars but also due to the decomposition of already present macro-infaunal and meiofaunal organisms. The introduced brittle stars are epifaunal and “lightweight” and should not cause a significant perturbation of the shallow sediment. The analysis of the foraminiferal vertical distribution presented in Langlet et al. (this issue) indicates that no major changes in the vertical distribution occurred during the experiment.

#AR1: It would seem that section 4.2 “methodological strategy of the study” would be more logically placed in the methods section, not the discussion.

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Authors' response: We think that the innovative aspects of our methods make it necessary to discuss them. Such a discussion is in our opinion better at its place in the discussion section than in the methods section, although we will try to shift the balance of that discussion more to the Methods, as suggested.

#AR1: Discussion: Do your oxygen consumption rate estimates (page 9257) take into account the probability that at least some of the taxa are able to use denitrification? How might this affect your estimates? Is your estimate a maximum?

Authors' response: At least some species of foraminifera are facultative anaerobes (Risgaard-Petersen et al., 2006, Piña-Ochoa et al., 2010) meaning that they may shift between at least two different metabolisms according to the redox conditions in the surrounding environment. If oxygen is available, foraminifera will probably use this electron acceptor to obtain energy; if there is no oxygen they may use nitrate as electron acceptor. Therefore, in our in situ experiment, we considered that foraminifera will first consume all available oxygen and can only switch to denitrification when oxygen is missing. As indicated in the text, the estimated oxygen consumption rates are only based on the foraminifera observed in the oxygen-containing sediment layers, where the use of alternative metabolic pathways is not very probable. However, it cannot be altogether excluded that anoxic microenvironments (with foraminifera with an anaerobic metabolism) exist in the oxic layer, and the estimate is therefore indeed a maximum estimate for the oxic layer. Conversely, some oxic microenvironments (due to bioturbation and inhabited by foraminifera with an aerobic metabolism) could also exist in deeper anoxic sediment intervals, compensating for this. Another, more important potential bias is the fact that we only considered the  $>63\mu\text{m}$  fraction. The relative contribution of foraminifera  $<63\mu\text{m}$  to the oxygen consumption rate is unknown and could potentially be important. Therefore, real oxygen consumption rates are probably higher than our estimates. In the revised version we will add a short paragraph discussing these points.

#AR1: How do you know that the newly available labile organic matter was consumed in the first month(s?) of the experiment (Page 9259)?

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Authors' response: As the experimental system is a closed system, only two sources of organic matter are available (the initial organic matter stock that is very low (Corg: 0.65 % wet weight; Koron et al., this issue) and the decay of dead organisms). Metzger et al. (this issue), who describe and discuss geochemical reactions observe an increased biogeochemical activity after 1 month, followed by much lower values in the "2 Months" chamber. These observations strongly corroborate the hypothesis of increased organic matter availability after one month, due to macrofaunal mortality, and complete consumption of these organic supplies before opening of the "2 Months" chamber. In the revised version we will add a sentence giving this extra argument.

#AR1: A list of the species that you found should be included in a table so that a comparison can be made with the RNA results, and so that readers don't have to refer to a separate paper in order to determine the composition of the assemblages you are referring to.

Authors' response: examples of species found living in all the groups identified by the molecular analysis will be added to the section 3.4. Several taxa from the order Rotaliida were found in the "10 Months" chamber (e.g. *Bolivina pseudoplicata* and *Bulimina marginata* s.l. for the major species), Textulariida (e.g. *Leptohalysis scottii*, *Eggerella scabra* and *Textularia agglutinans* for the major species) but the Monothalamiids were not analyzed in the present study.

Please also note the supplement to this comment:

<http://www.biogeosciences-discuss.net/10/C3586/2013/bgd-10-C3586-2013-supplement.pdf>

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Interactive comment on Biogeosciences Discuss., 10, 9243, 2013.

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