

***Interactive comment on “Structural and functional responses of harpacticoid copepods to anoxia in the Northern Adriatic: an experimental approach” by M. De Troch et al.***

**M. De Troch et al.**

marleen.detroch@ugent.be

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Dear editor, Dear referee,

we hereby provide you with our detailed answers to your remarks on our manuscript. The revised manuscript (including already the corrections suggested by the two other referees) is added in the ZIP file in the supplement. Our answers to your remarks can be found as a separate file in the ZIP document as well, and see also below. Don't hesitate to contact me in case of any further questions. On behalf of all co-authors I wish to thank you for your very valid remarks. With my best regards Marleen De Troch  
marleen.detroch@ugent.be

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Discussions Authors' reply to the Interactive comment on “Structural and functional responses of harpacticoid copepods to anoxia in the Northern Adriatic: an experimental approach” by M. De Troch et al. Anonymous Referee #3

Received and published: 8 April 2013 Replies of the authors are indicated in bold italics

**General comments** The contribution by de Troch et al. contains interesting information on meiobenthos enduring adverse conditions of anoxia in the Northern Adriatic. The study uses both classic approaches novel techniques and is of good scientific significance. There are results confirming well-known patterns (depth distribution) and results on the surprisingly small impact of short term anoxia, which make this a valuable manuscript. The quality of the presentation could be improved. This is mostly due to technical details outlined below. Also, too many details and small results are presented and discussed as if they were just as important as the main results: short term anoxia survival and reduced feeding during anoxia by copepods. Tables and figures are well designed. The presentation is good, however, the rather high number of citations, statistical information and the details given when referring to other studies, all together render some parts of the text difficult to read. My criticism mainly refers to specific points outlined below. However, I also consider this discussion as too long and not as streamlined as it could be. I further do think that there are quite a few citations already. This is why adding references in this review process should be avoided. Do not add citations suggested by reviewers without removing others. Please do think about cutting that list. - The authors appreciate it that the referee did a considerable effort to make suggestions to improve our manuscript. We agree that there are many papers cited, we removed 7 references in the revised version. On the other hand, since this study combines a field and a lab experiment we need to explain different techniques and thus more citations. All specific suggestions to improve the text were included, see further.

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#### Specific comments

Page 2486, Line 7-8: Change to: "The labeling technique resulted in isotope signatures ( $^{13}\text{C}$ ) of 17.29 ‰ for untreated and 8949.51‰ in  $^{13}\text{C}$  enriched cultures." (This is more easily read.) - The authors agree with the referee and this was changed in the revised manuscript (l.14-14 p.6 in the word document).

Page 2487: Line 14: for how long were the cores left before there was no oxygen any more? There are numbers on page 2493, line 15 indicating that concentrations fell to 10% of the initial value within 7 days. This means you witnessed a transient scheme. The aspect that anoxia was only short (in the experiments in the lab, too) should be made more clear. - The revised version of figure 2 (as requested by another referee) should clarify that anoxia was induced during 7 days i.e. cores were closed for 7 days. We only measured the oxygen concentration before and after closure of the cores. This implies that we indeed studied a short-term anoxia. We stressed this in the revised text by adding the word 'short-term' to l. 12 p.7. in the word document. The fact that this refers to 7 days is already mentioned in the description of the treatments in the paragraphs before. - This information only refers to the lab experiment. For the field data it was clearly stated that the EAGU was deployed for 5 days (see 2.2).

Page 2490: Lines 18 ff: It is a bit confusing to use the term "overall meiofauna density" and relate this to depths, i.e. layers in the sediment. I would think that overall density relates to a depth-integrated abundance like Individuals/10 m. Wouldn't the term 'depth-dependant abundance' (or density) be more appropriate? And to be exact: the data demonstrate that depth is the main factor determining a depth-dependant distribution! Well, is that astonishing? If it was not depth but organic carbon, oxygen availability or sulfide concentrations in the sediment that determined the depth distribution more than depth per se, that would be a result. That is, I completely agree with ref 1 that your findings on the depth as a major factor are presented in a way that attributes too much weight. It is fine to present the numbers, but the statistical proof that sediment depth is the main factor is not necessary here. The message is: no difference between

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anoxic and normoxic. And that is great. - First, we agree that overall meiofauna density is a confusing formulation. Actually we meant the total meiofauna density (so all meiofauna taxa) per depth layer. We revised and clarified this part by removing the word 'overall'. Actually the sentence, as it was initially meant, reads now as 'Overall, a two-way ANOVA on the total meiofauna densities (ind./10cm<sup>2</sup>) showed a significant effect of sediment depth (decreasing abundance with depth,  $p=0.002$ ) while the different oxygen treatments (normoxia/anoxia) interestingly showed no effect ( $p=0.05$ , Fig. 3A).' p.10 l. 13-16 in the word document. - The authors agree that this paper mainly focusses on the effect of anoxia but still we decided not to remove the information on the vertical distribution from the field experiment because of the following reasons: (1) the factor depth represents a level of variance in our sampling design that we can not neglect by applying for instance a one-way ANOVA, this would be wrong (2) our results showed that the effect of the sediment depth is stronger than the effect of anoxia (see Fig. 4 MDS), it is thus an important outcome of our study and indeed it probably points at other parameters related to depth, (3) we also showed that the anoxia effect differed in different sediment layers (see also remark 6 or referee 1, where he/she wants us to test this via a post-hoc test). This underlines the importance of reporting the vertical distribution in this study and (4) it is a standard procedure in meiofauna research to report vertical distribution, so this allows comparison with other studies,.

Page 2493: Results show an enormous pigment variation in untreated and treated cores. This patchiness in natural cores, especially given the small diameter of cores, is not uncommon. How do you deal with it? what does it do to your interpretation? - This could be an interesting point, however, we were not really after any change of chl a concentration over time, i.e. from T1 to T2. Since the diatoms were  $^{13}\text{C}$  prelabelled we mainly aimed to trace the uptake of them by the copepods rather than following their growth in the cores over time (from T1 to T2). The large variation in pigments is indeed probably due to patchiness in the cores. To answer the research questions of the present study it was essential that the addition of diatoms caused a significant difference with the treatments without extra diatoms. This was the case and this was

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also included in the discussion: 'Finally, it is possible that there was already a high amount of initial food present in the sediment and any addition of extra diatoms would not imply any significant difference between both treatments at the onset of anoxia. The contrary, however, was true because the Chl a concentrations increased significantly in the treatments with additional diatoms.' (p. 18 l. 12-15 in the word document).

Page 2494, line 10: "More specifically, the anoxic treatment with extra diatoms (T2AD) had a lower survival rate : : : than the one with diatoms". Why is there with? Should this read: without extra: : ? - This remark is correct, in the last case it should be without extra diatoms. The sentence was revised according to this remark: 'More specifically, the anoxic treatment with extra diatoms (T2AD) had a lower survival rate ( $66.9 \pm 5.8\%$ ) than the one without extra diatoms ( $80.3 \pm 21.5\%$ , T2A)' (p. 13 l.14-16 in the word document).

The fact that survival is reduced in combination of oxygen lack and diatom addition should be a more prominent thought. These are adverse effects, since additional carbon may intensify oxygen depletion. At least a settling bloom or eutrophication dependent carbon supply often times is responsible/adding to depletion situations.

It is nicely shown that feeding ceased after T1! Good!

In the Discussion Page 2495: the statement in line 15 ("contrasts with previous studies") seems to contradict those in lines 18 and 21. Please clarify. - Actually, lines 15-16 (l.15 p. 14 in the word document) mention that meiofauna decreases because of anoxia while the following lines say that they decrease but never disappear completely ('no complete mortality'). This part was revised as 'In the present field experiment, total meiofauna densities were not significantly affected by anoxic conditions. This is in contrast with previous studies that showed a significant decrease of meiofauna densities due to anoxia (Moodley et al. 1997; Travizi 2000). While studies on macrofauna revealed a peak in mortality at the transition from severe hypoxia to anoxia (Riedel et al., 2012), meiofauna – in general being more tolerant (Moodley et al. 1997) – decreased

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in density but may still be alive. Van Colen et al. (2009) created hypoxic conditions in a tidal mudflat for 40 days. While no macrobenthos survived, nematode diversity and abundances, for example, changed but no complete mortality occurred.' (l.10-17 p. 14 in the word document)

Page 2496: line 9 and 13 repeat the same issue. Please shorten. - This part was revised as 'By sealing a 50x50x50 cm volume off from the surrounding environment (Stachowitsch et al., 2007), the field experiment mimics the situation where water column stratification is the main cause of hypoxia, i.e. the isolation of bottom water from oxygen-rich surface water (Diaz, 2001). As this set-up caused a total cut-off of the food supply, eutrophication as an important factor in creating anoxia (Gray et al., 2002) was however neglected.' (l.28-32 p. 14 in the word document). The repeated sentence on the 'total cut-off of the food supply' in line 13 was deleted.

Page 2497: Lines 6-9 are repetitive of what other found. They do not help in the discussion of your findings and are somewhat superfluous text here. They should be omitted. Lines 21-24 could be omitted completely. - Lines 6-9 try to explain why copepods and nematodes respond differently. This part was revised as 'This difference in response can be due to phylogenetic constraints and lifestyle (Wetzel et al., 2001). Typically, a low oxygen demand in combination with a high surface:volume ratio enable some species to survive hypoxia/anoxia for extended times.' (l. 21-24 p. 15 in the word document). Lines 21-24 were deleted in the revised version.

Page 2499 line 19: replace "into" by "in". Also: which cores are you referring to? The ones from Gray 2002? - The word 'into' was replaced by 'in'. We refer to what we expected to happen in our experimental cores that received additional diatoms. In order to clarify this, the sentences were revised as 'Alternatively, as decomposition of organic matter (here diatoms) typically results in low oxygen concentrations (Gray et al., 2002), the cores with additional diatoms (T2AD) were expected to have lower DO concentrations and consequently lower survival rates than those without diatoms. This was however not the case in the present study as there was no significant difference

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in oxygen level in cores with or without extra diatoms.' (l. 16-21 p. 18 in the word document)

Fig. 6. This is a bit confusing. What about: Absolute and relative fatty acid composition of the sediment (0–3 cm) for treatments without extra diatoms (A, C) and for treatments with diatoms (B, D) - We agree that this read easier and the letters in the legend were therefore reshuffled as suggested.

Fig. 6. There is no figure 6B or 6C in my copy! - The referee is absolutely right. Also referee 1 pointed at this. We initially wanted to show also the further standardisation towards total uptake per individual and per unit copepod carbon. At the end we decided to remove these figures as they showed the same trend as in Fig. 7a. Apparently we forgot to remove/adjust the legends, the authors apologize for that.

Please also note the supplement to this comment:

<http://www.biogeosciences-discuss.net/10/C1683/2013/bgd-10-C1683-2013-supplement.zip>

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