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Biogeosciences
Discussions

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.

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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 the corrections suggested by the second referee) is added in the ZIP file in the supplement. Our answers to your remarks can be find as a seperate 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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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 #1

Replies of the authors are indicate in bold italics

Received and published: 9 March 2013 General comments This is a very interesting study combining a field and a laboratory experiment in order to determine the effects of anoxia on meiobenthic communities. The manuscript suits well within the focus of Biogeosciences and uses novel approaches (fatty acids, stable isotopes) to answer the questions addressed. The conclusions are well supported by the results and overall the paper is well written. However, there are a few flaws in the design and the presentation of the results which need to be taken into account before the manuscript can be accepted for publication.

Specific comments The main weakness of this study lies in the uneven design of the field and lab experiments, which makes the manuscript quite difficult to follow and the results at times irrelevant to the aims of the study. Differences between the two experiments include the different vertical sampling scheme, the fact that survival rates, diversity and chlorophyll was only measured in one (not always the same) experiment, different statical tests applied (e.g. two-way vs. one-way Anova's) and so on. To overcome this situation I think the authors should try to unify the analyses and prsentation of results between the two experiments by following the following simple steps:

âĂć Leave out the vertical distribution part of the study. Many such, more carefully designed studies, exists and most of them show what you have also found, namely

that the vertical depth plays a significant role in the distribution of meiofauna. Moreover, your aim, as seen in the Title, Abstract and Introduction was to investigate the short effects of anoxia and in my opinion you have done enough to support your case without the vertical distribution part (Le. clear effects of anoxia on copepods, effects of feeding behaviour etc.). Finally, since you have only detailed vertical distribution on the field experiment this part only complicates matters (see specific comment on ANOVA interactions) and confuses the reader. - The authors understand the remark of the referee that the removal of the data on the vertical distribution would simply the paper. First, we would like to explain that there was no info on the vertical distribution in the lab experiment because we needed sufficient material to obtain reliable stable isotope data. Therefore, we report only on the top sediment layer for this part of the paper. This issue is now explained in the Material & Methods section. 'To detect a reliable 13C/12C ratios in the tissue of the harpacticoids, a minimum of 15 μg C per samples is required. Therefore we used all live copepods from the 0-1 cm layer. There was insufficient biomass of copepods in the deeper sediment layer.' (p. 8 lines 2-4 in the word document) After all, we decided not to remove the information on the vertical distribution from the field experiment because of the following reasons: (1) it is a standard procedure in meiofauna research to report vertical distribution, so this allows comparison with other studies, (2) our results showed that the effect of the sediment depth is stronger than the effect of anoxia (see Fig. 4 MDS), this is an important outcome of our study that should remain in the paper and (3) we also showed that the anoxia effect differed in different sediment layers (see also remark 6, further in this referee report, where the referee wants us to test this via a post-hoc test). This underlines the importance of reporting the vertical distribution in this study. The specific remarks on ANOVA interactions were clarified as well (remark 11, see further).

âĂć Whenever possible do the same analyses and present the same results for both studies as this would be very helpful to assess whether the effects of anoxia are persistent in both the field and lab experiment or driven by experimental design artifacts. For example, diversity was only estimated and discussed for the field experiment. Survival

rate and chlorophyll was only measured for the lab experiment. Multivariate analysis was only done for the field experiment and so on. I understand that the two experiments may not have been designed together there- fore some analyses, such as survival rate or chlorophyll, are only possible for the lab experiment, however, other analyses such as diversity and multivariate can be performed for both. - First, survival rate and chlorophyll could not be estimated for the field samples as the cores were immediately stored in 4% formaldehyde in order to avoid any impact of oxygen in the anoxic treatment. Further, meiofauna extraction was performed with Ludox, so no survival rates could be estimated because of the formaldehyde and the Ludox. The main issue why certain analysis could not be performed for the lab experiment is the fact that all live copepods were needed for the stable isotope analysis. Moreover, it is impossible to put copepods in glycerine slides for identification prior to stable isotope analysis. In order to cope with this, we took 4 control cores at T0 and those were used to identify the harpacticoid copepods and report the community composition (see results, 3.2 first paragraph). Since these cores were not subject to any treatment it makes no sense to run a multivariate analysis on them as they only show the initial copepod community. Densities of higher meiofauna taxa were not estimated for the lab experiment as the paper focussed on the response of harpacticoid copepods (see title). In response to the referee's remark, we calculated the diversity for the copepods in the T0 samples of the lab experiment and the Hill indices were added to the revised text. 'Copepod family diversity was within the range of the diversity levels recorded for the field experiment (Table 1): N0= 6.8 $\pm$1.0, Ninf = 3 $\pm$0.8, H' (loge) = 1.7 $\pm$ 1.0. The higher Ninf is explained by the lower level of dominance of Cletodidae in comparison to their contribution in the field experiment (collected one year earlier).' (p. 13 lines 1-4 in the word document)

Technical comments 1. (Page 2484, Line 14). When where the normoxic samples taken at the beginning or at the end of the experiment? - The referee is right, this should be clarified. The sentence was revised as 'One day before the end of the deployment (i.e. at day 4) normoxic samples (3 replicates) were taken at ca. 4-5 m

distance from the chamber.' (p. 5 lines 12-13 in the word document)

2. (Page 2486, Line 20 and Fig. 2). Figure 2 and the way the experimental design is presented is a bit confusing. Try to make a better Figure by presenting the time paints linearly and sequentially and including To (start of the experiment). - Figure 2 was revised according to this suggestion of the referee, T0 was included. We agree that this clarifies the experimental design.

3. (Page 2487, Line 4). Ccores should read Cores - This typo was already corrected during proofreading for BG discussions.

4. (Page 2487, Line 16). Why did you use a different extraction method? This is rather strange since the cores were collected from the same area and I wonder if this had some effect on the results (see also point 12 below) - The main reason to avoid centrifugation with Ludox (was used for the field samples) was that we wanted to collected the animals alive for the stable isotope analysis. In that way, only copepods that survived the experimental lab treatment were used for the stable isotope analysis. Besides the fact that Ludox kills meiofauna organisms, it could also potentially impact the stable isotope signature. There is no carbon or nitrogen in Ludox that could directly affect the stable isotope measurements but there could be impurities in Ludox. Even more important is the osmotic effect of Ludox that can induce the leakage of components of low molecular weight. Also, samples should be prepared quickly in order to avoid leakage of 13C. This reason is now added to the revised text (p. 7 line 29-31 in the word document): 'Centrifugation with ludox was not applied as we targeted live copepods for the stable isotope analysis. Furthermore, ludox could impact the final 13C signal through its osmotic effect on components of low molecular weight.' We are expecting only some minor effects on the copepod densities as the decantation was repeated 5 times (see also answer to remark 12).

5. (Page 2490, Line 13). What were the initial H2S values? - The initial H2S was zero. This was clarified in the revised text (p.10 line 20 in the word document). 'H2S started

to increase soon after onset of anoxia, from 0 μM to final values reaching $\sim$ 29 μM.'

6. (Page 2491, Lines 4-5). Did you do any post-hoc tests? Did treatment differed in all depths or only at the surface? Its difficult to see from the figure. - This is indeed a very valid remark. We did some additional post-hoc tests (Tukey HSD). We found that indeed copepod densities (Fig 3b) were only significantly different between normoxia and anoxia in the top sediment layer. This information was added as 'In the latter case, the difference between normoxia and anoxia was only significant for the top sediment layer (0-0.5cm, post-hoc Tukey HSD, p=0.003).' (p. 11 Lines 3-4 in word document)

7. (Page 2491, Line 13). This sentence needs rephrasing as I do not understand it. You probably mean something like this: "For all these taxa there was both a treatment (anova ...) and a depth (anova ...) effect." - The referee is right that the formulation of this sentence is not clear and it was rephrased as suggested : 'For all these taxa, there was both a treatment (p=0.04) and a sediment depth (p=0.004) effect.' (p.11 line 11-13 in word document).

8. (Page 2491 , Lines 15-16). This is not true and I cant' figure out any such grouping on the MOS. Both the normoxia top layers (i.e. white and light gray triangles) and anoxia (Le. white and light gray circles) are far away and on both sides of the dashed line - This sentence actually refers only to the top layer (0-0.5 cm) and not to 0-1 cm depth. The 0.5-1 cm samples grouped indeed with the deeper layers. Therefore the text was corrected in this sense. We also added that there was still a considerable spread within the 0-0.5 cm replicates. The entire paragraph was revised as 'On the MDS plot on the relative meiofauna composition (stress=0.05, Fig. 4A), the top sedimentlayer (0-0.5 cm) grouped separately from the deeper layers (0.5-3 cm), which points to a strong effect of the sediment depth and no clear effect of anoxia on relative meiofauna composition. This separation was further confirmed by ANOSIM (R=0.651, p=0.001). A two-way crossed SIMPER analysis showed an average similarity in taxa contribution of 72.4% in the surface layers (0-0.5 cm) and 79.8% in the deeper layers (0.5-3 cm). Nematodes (60.0%, 95.4%, respectively in 0-0.5 cm and 0.5-3 cm) and

copepods (29.5% in 0-0.5 cm) contributed most to the dissimilarity between surface and the deeper layers.' (see p. 11 lines 14-22 in word document)

9. (Page 2492, Lines 10-14). Something is wrong with this sentence. Please rephrase. - I see now that something went wrong with the Latex format: The correct text was: '. . ..Cletodidae ($p<0.001$), Thalestridae ($p<0.01$) and Laophontidae ($p<0.1$). The MDS plot (stress=0.12, Fig. 4B) of the relative copepod families composition revealed a high similarity between the top layers (0-0.5 cm) of normoxic and anoxic samples, while the deeper layers showed a higher variability (i.e. sample points are more spread). The difference in relative family composition between surface (0-1 cm) and deeper sediment layers (1-3 cm) was supported by ANOSIM ($R=0.719$, $p=0.001$).' (see p. 12 lines 5-10 in word document) The bold text disappeared that is why you couldn't read it. I apologize for that. It is still correct in the word version of the manuscript.

10. (Page 2492, Lines 18 and 23). Try to be consistent. Sometimes you refer to the 0-1 cm layer (line 18) and sometimes to the 0-0.5,0.5-1 cm layers (line 23). You do not have a 0-1 cm layer for the field experiment. - We fully agree with the referee, sometimes the two top layers were interpreted as one, but we should note it correctly. This part has been revised as 'The difference in relative family composition between surface (0-0.5, 0.5-1 cm) and deeper sediment layers (1-3 cm) was supported by ANOSIM ($R=0.719$, $p=0.001$). SIMPER analysis showed an average similarity in family composition of 53.2% in the surface sediment layers (0-0.5, 0.5-1 cm) and 46.7% in the deeper layers (1-3 cm). Ectinosomatidae (44.9% and 41.5% in 0-0.5, 0.5-1 cm and 1-3 cm, respectively), Cletodidae (42.4%, 42.2%) and Miraciidae (6.7%, 13.6%) were the copepod families that contributed most to the dissimilarity between surface and deeper sediment layers.' (p. 12, lines 8-12 in the word document).

11. (Page 2492, Line 26 but also throughout the manuscript). I wonder if there were any interactions with these two-way ANOVA's. You should mention this explicitly because if you had interactions then you should have taken measures against them. - We agree with the referee. In a case of any significant interactions, these should be reported, if

not we didn't reported them. We checked the statistical analyses again and didn't find any significant interaction terms.

12. (Page 2493, Line 8-9). The To community appears to be quite different from the community of the field experiment (Le. different families are dominant). I would like to see this discussed. Could this be an effect of the different extraction techniques used or is it a matter of temporal change after a year? -The families Cletodidae and Miraciidae were found in high densities in both field and T0 cores (collected a year later). The samples collected the year afterwards by Grego et al (2013b, this volume) also showed a dominance of Cletodidae. For the other families there are indeed some differences that can be linked to interannual variability. Mainly the high relative abundance of Ectinosomatidae in both the normoxia and anoxia cores of the field experiment was not retrieved in the T0 cores for the lab experiment. The underestimation of this family can be due to the fact that these are mainly rather small species that are closely associated with sediment grains and that were not sufficiently extracted by the decantation method (see before for our arguments why not to use Ludox extraction for the lab experiment and the stable isotope analysis). These differences are now included in the discussion: 'The initial copepod community (at T0) differed slightly from the one reported for the field experiment (see before, collected one year earlier). The community was dominated by the families Cletodidae, Laophontidae and Miraciidae but a lower share of Ectinosomatidae was found while they dominated in the normoxic cores of the field experiment. This can be explained by interannual variability in the benthic communities. However, the samples collected the year afterwards by Grego et al (2013b, this volume) were also dominated by the family Cletodidae. Cletodidae were also found to dominate in the anoxic cores of the field experiment in the present study. Next to interannual variability, the underestimation of the family Ectinosomatidae can also be due to the extraction via decantation and not by means of centrifugation with Ludox (see field experiment). Since we wanted to use the live copepods, i.e. the individuals that survived the lab experimental treatment, the use of Ludox was not an option. Species of the family Ectinosomatidae are often rather small and closely associated with sed-

iment grains, it is plausible that they were not sufficiently extracted by the decantation method. In spite of these small differences in copepod family composition, there were no major changes in the overall diversity as the average number of copepod families in the T0 cores (6.8 $\pm$1.0) falls within the ranges reported for the normoxic (7.7 $\pm$ 0.6) and anoxic cores (6.0 $\pm$ 0.0) of the field experiment.' (p 17 line 5 in the word document and following)

13. (Page 2493, Line 13). I would be careful with your phrasing here as you can not say that anoxia was successful when you had (even low) evidence of oxygen presence. - We agree with the referee and rephrased the sentence as follows: 'The induction of anoxia yielded a significant decrease of the oxygen levels in the overlying water (one-way ANOVA, p<0.00001) from initial 6.6 $\pm$ 0.2 mg/l (T1N) and 6.4 $\pm$ 0.06 mg/l (T2N) to 0.58 $\pm$ 0.29 mg/l (T2A) after 7 days of closure of the core, independent of the addition of extra diatoms or not.' (p 13 lines 5-8 in the word document).

14. (Page 2493, Lines 17-end of paragraph). This paragraph is rather difficult to follow. Please try to make a Table with the Chi values including maybe also the other measured parameters. - This paragraph was rephrased as 'Chlorophyll a (Chla) concentrations were measured at time T1 and T2 and ranged between 0 and 90 μg/g. The addition of diatoms had a highly significant effect on Chl a values (p<0.0001) as samples without additional diatoms had <5 μg/g Chl a while treatments with extra diatoms had >35 μg/g Chl a. Because of the high variance in Chl a concentrations in treatments with additional diatoms (83.9 $\pm$ 52.2 μg/g (T1ND), 69.8 $\pm$ 33.3 μg/g (T2ND), 36.6 $\pm$ 24.0 μg/g (T2AD)), there was no significant difference in Chl a between the different time intervals, T1 and T2, (one-way ANOVA, p=0.35). Another pigment, Chlorophyll c2 (Chl c2), showed similar patterns as Chl a with max. 3.5 $\pm$ 4.2 μg/g (T1N) in treatments without diatoms and up to 8.7 $\pm$ 7.2 μg/g after adding diatoms (T2ND). In terms of carotenoids, the concentration of fucoxanthin ranged between 1.3 $\pm$ 0.1 μg/g and 2.0 $\pm$ 0.4 μg/g without additional diatoms and between 18.2 $\pm$19.2 μg/g (T2AD) and 39.7 $\pm$ 24.8 μg/g (T2ND) in treatments with extra diatoms.' (p 13 lines 9-22 in the word

document). The authors believe that the differences between the values are very clear but that there are not enough values to list them in an extra table.

15. (Page 2495, Lines 3-5). Something is wrong here. First of all, from the graph it seems that normoxia increased to about 1200 and not 952 as stated in the text. Moreover, in Figure 7 legent there is a statement that the Figure consists of (A), (8) and (C) but I got only one graph (probably only the (A) part) in my pdf copy. - The referee is absolutely right. 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 the legends, the authors apologize for that. The correct $\Delta\delta$13C are now reported in the text: 'Before the onset of the anoxia, the copepods were fed for 3 days with labelled diatoms, which resulted in the increase of their δ13C$\pm$stdev from -22.4$\pm$1.4‰ (T1N) to 276.9$\pm$192.8‰ (T1ND) ($\Delta\delta$13C=299.2$\pm$192.8‰ . In the normoxic treatments, a significant increase of $\Delta\delta$13C from T1ND (299.2$\pm$192.8‰ to T2ND (1281.5$\pm$667.6‰ was recorded (one-way ANOVA, p=0.03), indicating continuous feeding in normoxia (Fig. 7). In the anoxic treatments, food uptake ceased, with $\Delta\delta$13C values showing no significant difference between T1ND (299.2$\pm$192.8‰ and T2AD (138.6$\pm$43.0‰ (one-way ANOVA, p=0.16). Consequently, the $\Delta\delta$13C value differed significantly between normoxic and anoxic treatment (one-way ANOVA, p=0.014).' (p 14 lines 13-21 in the word document).

16. (2497, Lines 7-9). This sentence is incomplete as it misses a verb. Maybe you intended to have this sentence together with the previous one as one sentence? - This sentence was rephrased as 'Typially, a low oxygen demand in combination with a high surface:volume ratio enable some species to survive hypoxia/anoxia for extended times.' (p 16 lines 7-9 in the word document).

17. (Page 2497, Line 26). Please rephrase. Its no wonder you found effects at lower taxonomic level only for copepods since this is the only group you looked at lower level! - Correct, this sentence was rephrased as: 'At lower taxonomic level, there was a clear

effect of anoxia on the harpacticoid copepods' family composition.' (p 16 lines 25-26 in the word document).

18. (Page 2498, Line 1). "by see Grego ... " should probably read "but see Grego ... " - Corrected (p 16 lines 29-30 in the word document).

19. (page 2500, Line 27) The "a" in the "a for copepods" is a typo - Corrected as '(i.e. see Grego et al., 2013a, for copepods and nematodes)' (p 19 lines 28 in the word document).

20. (Table 1). Explain in the caption that this is only for the field experiment. However, I would also like to see the diversity values from the lab experiment. I do not understand why these were not calculated and discussed. Also, I would suggest to make the table a bit more easy to read by removing the second "Depth" column and by adding another row caption on top indicating the normoxic and anoxic part of the table. - The caption was revised as: 'Table 1. Average Hill's diversity indices ($\pm$ stdev) for (A) meiofauna taxa and (B) copepod family composition in the field experiment.' The lay-out of the table was revised and indeed it reads more easily now. Thanks for this suggestion! In response to the referee's remark, we calculated the diversity for the copepods for the lab experiment as well and the Hill indices were added to the revised text. 'Copepod family diversity was within the range of the diversity levels recorded for the field experiment (Table 1): N0= 6.8 $\pm$1.0, Ninf = 3 $\pm$0.8, H' (loge) = 1.7 $\pm$ 1.0. The higher Ninf is explained by the lower level of dominance of Cletodidae in comparison to their contribution in the field experiment (collected one year earlier).' (p. 13 lines 1-4 in the word document). These diversity levels were also further discussed. 'In spite of these small differences in copepod family composition, there were no major changes in the overall diversity as the average number of copepod families in the T0 cors (6.8 $\pm$1.0) falls within the ranges reported for the normoxic (7.7 $\pm$ 0.6) and anoxic cores (6.0 $\pm$ 0.0) of the field experiment.' (p. 17, lines 18-21 in the word document).

21. (Figure 4). What are the dashed lines? Why is not MDS done for the lab exper-

iment? The different gray symbols are difficult to distinguish. Maybe use numbers? - The dashed lines separate the 0-0.5 cm samples from the rest (in the A panel) and 0-0.5 cm and 0.5-1 cm from the rest (in the B panel). - We didn't aim to do a community analysis for the lab experiment, since only the copepods of T0 cores were identified (see also the second specific comment by the referee). The copepods from the other treatments were used for the stable isotope analysis. - Numbers instead of symbols would probably complicate the figure even more. The second referee had no remark on the symbols, but still we made an attempt to clarify the fills of the symbols.

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/10/C572/2013/bgd-10-C572-2013-supplement.zip

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