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Interactive comment on “CellTracker Green labelling vs. Rose Bengal staining: CTG wins by points in distinguishing living from dead anoxia-impacted copepods and nematodes” by M. Grego et al.

M. Grego et al.

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We are happy to take up the editor’s and reviewer’s offer and re-submit the revised version of our manuscript titled ‘CellTracker Green labelling vs. Rose Bengal staining: CTG wins by points in distinguishing living from dead anoxia-impacted copepods and nematodes’ (Grego et al.). We have taken a close look at the 3 reviewer’s comments and are happy that they are so thoroughly positive. We have been able to address almost every point they made, which helped to improve the manuscript in the sense suggested in virtually every case. The items outlined below are listed in the order

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given by reviewers 1-3 and refer to the lines in the original manuscript.

[Anonymous referee #1's comments]:

I found the manuscript by Grego et al. to be well-designed and well-written. The study is relevant to future marine community ecology studies that focus on meiofauna, foraminifera, or even bacterial. In particular, studies of hypoxia or other short-term disturbances should consider the use of CellTracker Green to distinguish living vs. Dead metazoan meiofauna. The authors clearly delineate the advantages and disadvantages of each staining method. The authors present an adequate discussion of their results and the conclusions are supported by the data. The statistical analyses and discussions thereof are accurate. I could make minor editorial comments, but there is no reason that the manuscript can't be published in its current form without revision.

- We appreciate the reviewer's enthusiasm for the exciting possibilities that the CTG approach opens up in "meiofauna, foraminifera, or even bacteria" studies.

[Anonymous referee #2's comments]:

This is a very well written manuscript in terms of structure, extent and clearness. Its findings are relevant to all meio/micro-benthologists who consider experimental work in the future. I read it with great interest. The introduction is concise and relevant. The experimental setup and execution is clearly described and adequate for the study. It meets a standard that is impeccable. The laboratory analyses and statistics are well described and performed and support the author's conclusions. The claims the authors make are adequately discussed. In general, the methodology is clear and reproducible and the literature is treated in adequate and comprehensive way. I recommend the manuscript for publication in Biogeosciences after following minor considerations:

- My co-authors and I thank reviewer 2 for his/her time and the positive, constructive comments.

- 2859 L6: the reference Diaz and Rosenberg, 2008 is now included in the reference

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list

- 2860 L11-13: sentence now modified as suggested.
- 2860 L16: sentence now modified as suggested.
- 2860 L19-20: deleted as requested..
- 2860 L24: corrected to "deep-sea"
- 2860 L27: deleted as requested
- 2861 L6: "treats" now replaced with "describes"
- 2861 L12: The sentence was modified as suggested and now reads: "In oxygen minimum zones, for example, nematodes are found in higher densities (Veit-Köhler et al., 2009) compared to surrounding well-oxygenated sediments, presumably benefitting from high food supply and low predation and competition pressure (Neira et al., 2001). *this reference was added to the reference list: Neira C., Sellanes J., Levin LA., Arntz WE. 2001. Meiofaunal distributions on the Peru margin: relationships to oxygen and organic matter availability. Deep-Sea Res Part I 48: 2453–2472
- 2861 L17: "toxic" deleted
- 2863 L20: One extra replicate per treatment (CTG and RB) was taken as a backup, to avoid a shortage of a replicates in case of any accident during sample processing. Especially in the case of CTG, one extra replicate ensured us, since we were applying the CTG technique for the first time for meiofauna study." We have now added information to clarify the choice of replicates: Line 21: "The two remaining cores were taken as backup cores."
- 2863 L24: You are right, CTG is a fluorescent probe. The sentence has been corrected
- 2870 L14: "microbes" corrected

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[Anonymous referee #3's comments]:

The paper compares the efficiency of the use of two methods of staining to separate dead and living organisms in normoxic and anoxic sediments. The two methods are Rose Bengal and CellTracker Green and the selected organisms are nematodes and copepods. The authors conclude that the CTG appears more efficiency than RB to discriminate dead and living organisms. The results presented here are based on an experiment carried out in the Northern Adriatic Sea, using a benthic chamber to create the anoxic conditions. I find the paper very interesting and well organized.

- We thank the reviewer for his/her detailed and constructive comments. We reworked the respective parts of the manuscript and thereby have, we hope, increased the clarity of the manuscript.

[Comment]: My main concern is on the samples treatment. CTG has been incubated for 12h and then samples have been fixed while RB has been added into fixed samples. In my opinion, samples should be treated following the same procedure since the aim of this investigation is to find the best method to discriminate dead and living organisms. So also samples with RB should be incubated for 12h and then fixed.

- This is a very good point and indeed, from a strict experimental point of view, it may be better to treat all samples in exactly the same manner. However, in this case the two treatments differ fundamentally in their mode of function. While the CTG must be incubated because the living organisms have to take it up, this is not the case for RB. Moreover, following the standard procedure, RB is always added to fixed material and never incubated with living organisms (Higgins and Thiel, 1998; Somerfield et al. 2005). As this is the first study which compares these two treatments in meiofauna, we chose to apply these standard methods. Based on our long-term experience in meiofauna sample treatment, we believe that a RB incubation would not have fundemantally changed the results or our conclusions; we thus trust and hope that our approach is deemed acceptable.

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[Comment]: I find also not correct that the authors used this experiment to “examine fine-scaled and short-term disturbance phenomena” since the experiment is based on a comparison between meiofaunal assemblages from anoxic and normoxic sediments collected at the same time. The experiment does not include different sampling times that describe the evolution of the system from normoxic to anoxic conditions. I think that this point should be clarified in the abstract, in the introduction and conclusion.

- Thank you for that comment. This point is probably more a misunderstanding of our intended meaning of “fine-scaled and short term”. We simply wanted to point out that, as opposed to most approaches that quantify abundance/survival/mortality at some time after hypoxia/anoxia, the CTG method can actually be used to more precisely follow and quantify organism’s death and degradation during such an event, potentially even at hourly intervals. Nonetheless, to avoid confusion we have rephrased/removed the formulations from the abstract and conclusions. The sentence in the abstract was rephrased and reads now: “For monitoring and biodiversity studies, the RB method might be sufficient, but for more precise quantification of community degradation, especially after an oxygen depletion event, CTG labelling is a better tool.”

- In the conclusions, the modified sentence is: “If the task is to examine survival of animals in hypoxia, anoxia or other types of disturbance,... ”

- This was also clarified in the introduction, so that it is clear now that there was no treatment in between the normoxia and anoxia samples. Normoxia represents T0, and anoxia T1- after 5 days of chamber closure. Therefore we rephrased and added in the introduction/aims the original sentence from “Here we test whether the CTG-labelling is a more accurate method than the widely used RB-staining for hypoxia/anoxia studies, especially because here the changes in copepod and nematode community composition can occur in the course of hours“ to: “Here we test whether the CTG-labelling is a more accurate method than the widely used RB-staining for quantifying living meiofauna in hypoxia/anoxia studies. The primary goal of the work described here was to compare harpacticoid copepod and nematode density in normoxic sediment samples

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with those in anoxic samples. Anoxia was experimentally induced by means of...

[Comment]: Specific comments: Abstract Line 14, delete “Surprisingly”, since it is known that RB stained all material containing proteins, in the case of nematode, the presence of different material on the cuticle can contribute to the overestimate of the living organisms.

- The term “surprisingly” was removed throughout the text. Furthermore, we added the sentence in the discussion: page 8, line 241: “As RB stains all material containing proteins, the presence of different material on, for example, the cuticula of nematodes can contribute to an overestimate of living organisms.”

[Comment]: Lines 15-18, I am not sure that the experimental set up allows to “resolve the course of events”. In the present investigation anoxic and normoxic conditions are compared. There is not an analysis between a T0 and T end of the experiment.

- Abstract, L 19-22: the sentence has been modified to: “For monitoring and biodiversity studies, the RB method might be sufficient, but for more precise quantification of community degradation, especially after an oxygen depletion event, CTG labelling is a better tool.”

[Comment]: Introduction Pages 3-4, lines: 94-96, At the end of the introduction the aim of this investigation should be better explained. In particular in the present form, the aim seems to be related to “the changes in copepod and nematode community composition can occur in the course of hour”. Again, the experimental set up allow to investigate the differences between anoxic and normoxic sediments but do not allow to discriminate differences during the changes in the environmental conditions.

- 2861 L28-31: The sentence was modified and reads now: “Here we test whether the CTG-labelling is a more accurate method than the widely used RB-staining for quantifying living meiofauna in hypoxia/anoxia studies. The primary goal of the work described here was to compare harpacticoid copepod and nematode density in normoxic sedi-

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ment samples with those in anoxic samples.“

[Comment]: Conclusion Page 10, lines: 334-335: please rephrase. I would suggest to remove references from the conclusions.

- References were removed as suggested and the sentence rephrased. It reads now: “If the task is to examine survival of animals in hypoxia, anoxia or other types of disturbance, and a binocular microscope with UV production light and filter- is available, then we highly recommend the CTG technique. This also echoes the recommendation of foraminiferan researchers.”

- Tables 1 and 2: p-level value was corrected.

We believe we have addressed all the points made by the reviewers and look forward to seeing our manuscript in Biogeosciences.

Sincerely,

Mateja Grego and co-authors

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