

Interactive comment on “Variable metabolic responses of Skagerrak invertebrates to low O₂ and high CO₂ scenarios” by Aisling Fontanini et al.

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General comments The topic of the present work is interesting and the authors try to make a correlation between the obtained data and the environmental characteristics of habitats. However, my concern is the period of exposure to particular stressors, which may determine and the range of tolerance of marine invertebrates to environmental changes. For example, it is reported (page 8, line 43) that invertebrates from this ecosystem which showed little or no mortality in the presence of both stressors, reflects the range of conditions in the habitats these organisms occupy. However, mortality is depended on several factors including reproduction period, body size, etc. Moreover, it is depended on the period of species exposure to stressors. Also, a key point for the species to withstand stressful conditions for long term is their ability to keep a stable

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energy turnover since. Such metabolic responses and patterns determine and their thermal limits. Even more some species live at the edges of their range of thermal tolerance. Thus, long-term experiments might help further not only in estimating species' ability to withstand stressful conditions but to make a better correlation with the future climate projects. I consider that the authors should take into their consideration the above points and to reconsider the interpretation of some obtained data. I agree with author's statement about the complexity of stressors and the challenge of predicting how global stressors will affect marine ecosystems in the future. Reply: We agree with the comments in regards to longer-term experiments, however it is beyond the scope of this work. Our research questions were targeted at being able to detect responses to short-term and acute environmental changes that may occur suddenly as part of eutrophication events. We chose future climate change targets for O₂ and CO₂ levels as they are realistic representations of what will come in the future, or in some cases might already occur nowadays.

Specific comments Page 2, line 8. This metabolic control. . .could be changed to The involvement of metabolic processes in the regulation of the pH in coastal water is... .. Reply: Changes made as suggested. It now reads “The involvement of metabolic processes in the regulation of pH in coastal waters is particularly evident when eutrophication stimulates algal blooms, leading to increased sedimentation of organic matter, subsequently degraded through microbial respiration, thereby consuming O₂ and releasing CO₂ (Conley et al., 2009)”.

Page 2, line 15.although the combined stress from depleted O₂ and high CO₂ is likely to provide a significant challenge to coastal invertebrates and less mobile species... could be changed to although the synergistic effect of O₂ depletion and CO₂ accumulation is likely to provide a significant challenge to coastal invertebrates and mostly to sessile species. Reply: Changes made as suggested. It now reads “Whereas scientists have acknowledged this coupling over decades, the impacts of these two pressures have continued to be studied in isolation, although the synergistic

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effect of O₂ depletion and CO₂ accumulation is likely to provide a significant challenge to coastal invertebrates and mostly to sessile species.”.

Page 2, lines 24-26. There are many invertebrates tolerant to hypoxia (e.g. mussels). Thus, the authors should be focused on these species which rather are less tolerant (e.g. benthic invertebrates). Reply: As the aim of the study was to test the combination of two stressors, we also used “tolerant” species to see how they react to the combination of the two stressors hypoxia and elevated pCO₂. Moreover, it has been shown that responses are highly species specific and not taxa-related (see Fabry, 2008; Malakoff, 2012; Calosi et al., 2013).

Page 2, line 37. I would prefer synergistic instead joint Reply: We made the change as suggested.

Page 2, line 38. ..future levels of what I consider that the two last paragraphs should be reorganised and rewritten in such a way so the firstly the authors to be reported at several hypotheses and secondly at their aims Reply: We reorganized the paragraphs as suggested.

Methods and Materials 1. Merge the two first paragraphs Reply: Changes made as suggested.

2. Make clear, when saying history, whether the reported environmental characteristics are long lasting. It is very important since species experiencing such environmental changes in their life cycle may have adapted to such environmental changes by developing the corresponding cellular and physiological mechanisms. Reply: This has been re-worded to show that we are referring to natural and sustained seasonal events which occur in winter and can be exacerbated by nutrient enrichment. It reads now “Both natural and anthropogenically enhanced hypoxia occur within the fjord when enrichment is high and seasonal water exchange over the sill is slow (Josefson and Widbom, 1988; Arneborg, 2004)”.

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3. Report which of the examined rocky species are exposed or not to air because of tide. The latter characterizes sessile species tolerant to hypoxia. Reply: There is no real tides in the fjord where we collected the specimens. The seawater level can change by a few dm (less than 1 meter) depending on atmospheric pressure, winds, etc. Among the tested species, only *Littorina* sp. and *Mytilus* sp. can be occasionally be exposed to air.

4. Change Metabolic response to Metabolic rate or Oxygen consumption. Metabolic responses usually is referred when we examine the metabolic patterns (e.g. enzyme activities, metabolites etc) Reply: Changes made as suggested.

5. Page 5, line 8. For the readers describe briefly the physiological meaning of term respiration index. Reply: We feel that this has been described in the following sentences. But if the editor wants us to describe it in a different way we will add a description.

6. Page 5, lines 15-21. The experimental procedure for determining the oxygen consumption should be written in details. For example, chamber volume, was it the same for all species examined? Reply: We updated the formula in the manuscript to mg L⁻¹ O₂ min⁻¹ L⁻¹ g DW⁻¹ as the volume of the glass chamber was included in the calculation. Thus, we don't feel the need to report all the chamber sizes in the manuscript. But if the editor is of the opinion that those data (mean SE for each treatment) are essential for the manuscript, we will off course add this information.

Also report the temperature, salinity and pH of water. It is very important to report the period (hours) of experimental procedure since under a particular level of PO₂ metabolism shifts from aerobic to anaerobic and this point is species depended. Reply: The water for incubation had the same values as the experimental aquaria and held in the same room so the temperature and salinity would be the same as reported in Table 2. Incubations lasted a maximum of 5.5 hours, to make sure there is some oxygen left in the glass chamber. None of them reached 0.0 mg L⁻¹ oxygen. We added the max.

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incubation time to the manuscript but don't see the need of reporting time (minutes) in detail. But if the editor is the opinion that those data (mean SE for each treatment) are essential for the manuscript, we will of course add this information.

7. Page 5, line 23. Ratio of what? Reply: Changes made as suggested and it reads now "The response ratio of the respiration rate. . .".

Results 1. Respiration. Report the consumption of oxygen rate for each examined species and give possible differences between each other. Reply: The data are provided in Table 3 (mean SE).

2. Give more information the differences or not for the oxygen consumption for each species at each treatment Reply: The data and results of statistical tests and GLM are shown in Table 3. We don't feel the need to report all of them twice and mention them again in the text of the manuscript. But if the editor is of the opinion that it is 100% necessary we will make the change as suggested.

3. In general the results should be rewritten in such a way so to be more clear what is happening in each species at tested treatments and whether differences were recorded from species to species. Reply: Our research questions were more targeted towards the differences between treatments for each species and how their habitats may have played a role.

Table 3. In the column day it is marked 3/6, 3/5 etc. In the legend it is reported pooled data where we had 3 and 6 days. Thus the number 4, 5 2 what do they mean. Reply: These were days of measurement, that were pooled. We have altered the wording to reflect this (in Table 3 and methods).

Discussion Page 7, line 29-30. It is unclear what the authors report. Reply: Changes made for clarity. It now reads "The community in this area has already been sieved of species vulnerable to low O₂ concentrations due to a history of hypoxia and even complete anoxia within the last four decades (Nordberg et al., 2000; Polovodova et al.,

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2011):".

Page 7, line 32-33. It is very important to report whether such changes in pH regard fluctuations or permanent changes. In the first case the organisms face waves of such changes and how long such waves last. Reply: These just represent fluctuations. This sentence has been reworded to reflect this difference.

Page 7, line 36-37. Community of what? Rewrite the sentence (line 37-39), since it is unclear what it is meaning. Reply: Changes made as suggested. It now reads "Exposed *A. filiformis* live in sediment burrows that experience much lower oxygen and higher pCO₂ than surrounding water which intensifies with depth (Hu et al., 2014). *A. filiformis* have been shown to withstand a pH of 7.0 and O₂ levels below 2.0 mg L⁻¹ and experience no mortality (Hu et al., 2014). Hence, the species tested here already has O₂ and pH values comparable to those used as treatments here, particularly for infauna, such as *A. filiformis* and *B. lyrifera* which appear to be exposed to low O₂ and pH conditions on a regular basis."

Page 9, line 16. Responses . . .which responses? Reply: Changes made as suggested, it reads now "Respiratory responses".

Page 9, line 1-2. Do you know how long these events last? Is it an acute environmental change or long-term change? Reply: There is an overall trend towards decreasing oxygen over time in the Fjord (based on foraminifera populations) however hypoxic events can vary in duration. There is also a seasonal trend of decreasing oxygen over winter months before new water comes into the fjord.

Page 9, line 10. It could be nice if the authors could support such adaptive responses, genetically determined, by reporting differences from individuals of the same species but from different populations habiting environments differing in the tested abiotic factors. The observed responses in the present work may regard phenotypic plasticity which may be observed and in individuals from populations living in other environments when treated similarly. Reply: We were limited in time and logistics, thus there

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was no option for us to test different populations of the same species. But we agree that this should be taken in consideration for future experiments to compare if there are differences in responses depending on populations, water quality and conditions the individuals experienced previously.

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