

## ***Interactive comment on “Variable metabolic responses of Skagerrak invertebrates to low O<sub>2</sub> and high CO<sub>2</sub> scenarios” by Aisling Fontanini et al.***

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Received and published: 25 January 2018

Fontanini and co-workers investigated the respiratory responses of a range of invertebrates to short-term changes in oxygen and CO<sub>2</sub>. The paper gives some interesting insights which revealed the highly variable responses of invertebrates from a single area towards future scenarios. This reveals that e.g. general ecosystem models based on few species are not yet reliable. Therefore, this study highlights the need for more experimental work. Currently the manuscripts lack some important experimental details which make it complicated to judge the quality of the measurements. For instance what was the rationale behind measurements on day 3 and 6 and were any differences observed? Considering the strong physiological differences between the investigated phyla e.g. a crustacean and a tunicate or echinoderm it is not intuitive why the animals

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were grouped according to the habitat and not according to the lifestyle in figure 1. This could mask certain significances. Reply: The decision about the duration of the experiment was based on a meta-analysis by Vaquer-Sunyer & Duarte (2008) which found the median lethal time (LT50) for over 400 studies to be just over 5 days. Therefore, we felt we should be able to detect a sub-lethal response such as respiratory changes within that time-frame. Moreover, as we wanted to measure the respiration rate, we had to make sure that the individuals were still alive. We have added a statement to the introduction to highlight this. The habitat background was the same for the species collected from the same habitat. Most of the tested species were echinoderms and gastropods. As for crustaceans, bivalves and tunicates, we just had 1 species each and thus didn't see the need of grouping per taxa.

I have a couple comments and the MS would benefit from some careful corrections thus I recommend major revision. Specific comments Page 1 Line 25 that the responses of respiration of the respiration – please change Reply: Changes made as suggested.

P 2 line 6 please change to acidic water Reply: We changed the phrase and it is now reading “leading to a decreased pH” as we try to avoid the term “acidic water”.

P2 Line 7 what do you mean by ‘Control’? more precisely speaking: the biochemical processes which change seawater pH? Reply: Yes, we were referring to the biochemical processes and relationships that may cause pH to fluctuate over various temporal and spatial scales, drawing particular attention to the role of metabolism. This sentence has been altered to better reflect this. The sentence now reads “The involvement of metabolic processes in the regulation of pH in coastal water is particularly evident when eutrophication stimulates algal CO<sub>2</sub> for marine organisms reach further than the highly documented impacts on calcification rates (Doney et al., 2009).”.

P2 Line 18 Internal is not a precise term, intra- and extracellular pH regulation are two completely different processes. I assume you refer to extracellular pH as intracellular pH is commonly well regulated? Please specify. Reply: Yes, we agree and changed

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the phrase to “extracellular acid-base regulation”.

P2 Line 20 Hemoglobin is not common in invertebrates which this study is focused on. Reply: We have removed the reference to haemoglobin as well as the definition of hypercapnia as we do not investigate the impact on blood fluids within this study.

P3 Line 31 what is meant by ‘a history of North Sea upwelling’? commonly observed? Reply: Salinity at the surface of the fjord can change by 10psu in the summer months as salty water originating from the North Sea comes to the surface. We have decided to remove this sentence as the water we used during experiments had a stable salinity for all animals.

P3 line 3 and following? Where the salinity similar at surface and bottom of the Fjords as all animals were exposed to the same high salinity during the acclimation phase? Reply: The Sven Loven Centre has three water inflows from different depths in the Fjord. We chose to use North Sea (deep water) as animals from shallow environments are able to cope with the salinity due to the aforementioned upwelling events. As we have removed the reference to summer upwelling, we have also removed the reference to North Sea water from this sentence. This will have minimal impact as we have stated the salinity over the course of the experiment in subsequent sentences.

P4 Line 3 and following Based on this paragraph, *P. bernhardus* was the only species which was fed during the experiment? Is there any specific reason for this decision? Reply: Reply: This decision was based on advice from the lab technician in charge of looking after animals at the facility, which considerably experience in maintaining the organisms tested here. Given the short exposure periods, and the fact that most individuals are filter feeders, there was no need to add food to the aquaria. The crustacean *P. bernhardus* on the other hand is a carnivore and was fed ad libitum before the experiment started. We added the sentence “No animals were fed during their experimental period” to clarify.

P4 Line 8 and following Several species and sometimes specimens were kept in the

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same aquaria for logistic purposes? However, can you exclude that a number of co-variable influenced respiration rates similar to the observed abnormal mortality in one tank? Reply: Respiration rates of animals were measured individually in glass chambers, where no other organisms were present. All aquaria had the same 'mixture' of organisms at the same time, so we would expect to see the impact of co-variables across all treatments. Moreover, we made sure no predator and preys were in the tanks at the same time to exclude stress due to their presence.

P4 Line 16 and following The animals were kept in closed systems without any waster exchange. Did you check the water quality in order to monitor potential accumulation of waste products due to metabolism and mortality? Reply: Should that read Page 5 Line 16? As described on Page 5 Line 1, we replenished the water in the aquaria with a continuous flow of water. For the incubations to measure the respiration rate it was essential to keep the glass chambers/containers sealed as we measured oxygen at the beginning and end of the incubation. Any water-exchange would have influenced and changed those measurements.

P 5 Line 15 and following As respiration was only response variable measured in this study, more detailed information needs to be provided such as: Volume of the containers, did you control for a linear decline of oxygen concentrations? In particular, as this is the focus of the study, how much did oxygen decline during the incubation? Strong declines would severely affect the study concept. Reply: Oxygen consumption has been updated to  $\text{mg L}^{-1} \text{O}_2 \text{ min}^{-1} \text{ L}^{-1} \text{g DW}^{-1}$  as the different size of glass chambers was accounted for when calculating the respiration rates.

P 4 line 34 Why did you use two different pH meters and what differences did you observe? Reply: The Metrohm 827 pH meter was actually used to take daily point measurements and is shown in the information in the results section. The data logger was used as a reference for us to see what was happening overnight, but could only be placed in one tank and so has not contributed to any data reflected in this paper. We have removed the reference to the data logger.

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P 6 line 7 Here you state a target of 1000 ppm whereas it is 1300  $\mu\text{atm}$  in the M&M, even if target and measurement were not identical, the target should be uniform. Reply: Changes made as suggested.

P6 Line 37 A non significant response may only called a 'trend towards' Reply: Changes made as suggested.

P 7 line 7 the experiment did not last long enough to draw any reliable conclusion on survival rates Reply: As shown by Vaquer-Sunyer & Duarte (2008)'s meta-analysis that 90% of 282 studies experienced LC50 at 4.6 mg O<sub>2</sub> L<sup>-1</sup> with the mean LC50 for all organisms at 2.1 mg O<sub>2</sub> L<sup>-1</sup>. The median LT50 (460 studies) was 117 hours or nearly 5 days. We therefore still believe that the high survivorship of organisms over 3-6 days in the low O<sub>2</sub> treatments indicates a tolerance (acclimation or adaptation) to these conditions and is worthy of noting. Nevertheless, we added the phrase "short-term" to the sentence and it reads now "The Baltic species tested were highly resistant to short-term hypoxia and high CO<sub>2</sub>, alone or in combination, as they experienced very high survival rate across treatments in the relatively short-duration experiment reported here".

P7 line 14 To support this hypothesis you need to add a reference which documents higher mortality for populations from habitats with less abiotic stress Reply: In this particular line (and following), we make a suggestion and already added references.

P7 line 17 and following Please consider that the RI hypothesis and in particular the definition of an exact threshold is still under debate: <https://www.biogeosciences.net/10/2815/2013/bg-10-2815-2013.pdf> Reply: We have attempted to use Brewer and Peltzers RI's to test its ability to predict marine responses to O<sub>2</sub> and CO<sub>2</sub> and state in the discussion that we feel it did not hold predictive power in the context of this experiment. We hope this may contribute to the ongoing discussion.

P8 Line 37 Even though calcified structures and the calcification process might be

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affected by undersaturation it is not clear why this should be detectable in the rates of aerobic metabolism Reply: We agree and changed the phrasing. It now reads “Hence, the RI does not hold predictive power on the effects of hypoxia and/or pCO<sub>2</sub> on the species tested here, which seemed best predicted from consideration of the ranges of O<sub>2</sub> and CO<sub>2</sub> they experience in their habitat.”.

P9 line 6 Hypoxia is necessarily always coupled to elevated CO<sub>2</sub> Reply: Changes made as suggested. It now reads “and is, therefore, coupled with elevated pCO<sub>2</sub>”.

Table 3 please give the unit for respiration rates Reply: Changes made as suggested and added the information requested.

Some references are either missing in the text or in the reference list e.g. Grans or Gräns? et al. is not in the list Reply: We checked the reference list and made the changes as suggested.

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Interactive comment on Biogeosciences Discuss., <https://doi.org/10.5194/bg-2017-321>, 2017.

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