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Comment

## ***Interactive comment on “The effects of intermittent exposure to low pH and oxygen conditions on survival and growth of juvenile red abalone” by T. W. Kim et al.***

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This manuscript is based on an excellent idea: study the impact of upwelling of low pH and oxygen waters on juvenile red abalone. This leads to the key question of the biological response to short term exposure (<24h) to challenging environmental conditions.

This is highly relevant in the context of global climate changes (warming, acidification, deoxygenation). Changes that are occurring on top of the natural variability and short term fluctuations are often neglected in experimental designs.

If authors can answer the following questions, this can be an influential paper (nice

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“proof of concept”).

The dataset is interesting but more information on methods is needed to fully evaluate its potential. Moreover, new analyses are needed. I suspect that a new analysis may change the conclusions (the manuscript should then be rewritten accordingly). See below for detailed comments.

1. TERMINOLOGY: Two different experiments were performed:

(i) “short term”: an experiment lasting 11(?) days with 2 x 3h and 1 x 6h exposure to treatment waters (ii) “long term”: an experiment lasting 26(?) days with 2x24h exposure to treatment waters.

The terminology “short” vs “long” is rather confusing. It took me some time to understand if you were relating to the duration of the experiment (11 vs 26 days) or the duration of the treatments (3-6h vs 24h). Neither 11-26 days or 3-24h could be considered as a real “long term”. I suggest using another terminology. A number of parameters are changing between the two experiments (e.g. number of exposure to treatments (2-3), the duration of the exposure to treatment (3-24h), total duration of the experiment (11-26 days), maybe size of the juveniles, etc.)

As a consequence, I suggest using a more general terminology such as “experiment 1” and “experiment 2”. You can then discuss the proximal factors responsible for the difference between the two experiments in the Discussion.

This should be changed in the whole manuscript.

2. ADDITIONAL INFORMATION is needed in Materials and Methods.

2.1. It is quite difficult to understand the experimental design for the two experiments (you have to look for the information in different parts of the experiments).

2.2. It is critical to provide more information on how pH/DO were manipulated. In the present version, it is not possible to have a clear idea on the dynamic of change

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and the level of variability through time. One easy and elegant way to solve this would be to make a figure with the evolution of pH through time in the different treatments/experiments. That would solve both points 2.1. (would clarify the experimental design if you add information on sampling points on this graph) and point 2.2 (dynamics of change and variability). Some additional information on the dynamic of changes during upwelling in the field would also be interesting (not only duration but how fast it changes and how constant it is).

2.3. It seems that both "dead" and "alive at the end of the experiments" individuals are used to calculate Growth rate (GR). However, the two groups should not be considered in the same way in the analysis. Death is likely to be associated to high energy costs that can impacts GR. This could explain why you see negative growth (Figure 2). A nice way to present this would be to use scatter graphs instead of bars and use different color codes for dead and alive.

2.4. It is explained that number of dead animal is measured but not how mortality is calculated (and there is no unit on Figure 1).

2.5. The method to measure GR (line 160, REM: all line numbers refered to the submitted version of this manuscript, before edition to BGD) is assuming a linear growth with time and no effect of the initial size. This is unlikely for juveniles that often grow exponentially and then: (i) the growth over a given period of time is highly dependent on the initial size; (ii) for same initial size, the GR is different when calculated over different period of time (e.g. dead vs alive). This can introduce some strong noise in your dataset and you should prove that this is minimized (e.g. using regressions).

Other information needed: - Line 81, please provide more information on the origin of the juvenile used: what is the original population (before being cultured)? What were the culturing conditions (e.g. temp, pH) in the abalone farm? These could be important parameters to interpret the results (e.g. artificial selection in culturing facilities, "well fed" juveniles compared to what could happen in the field, etc.) - Line 85, is food

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provided “ad libitum”? See the recent paper of Thomsen et al. 2013 on the key role of food in modulating bivalve response to ocean acidification. - Line 87, some information on conditions (temp, pH, etc.) at HMS. - Line 91, precise the pH scale (I guess it is total scale? If yes, maybe use pHT?) - Line 110, precise pH scale, calibration method and frequency of monitoring. - Line 123, 140 what is “SM”? - Line 128. “dead abalone, if any, were transferred to vials for later measurement”. What measurement? Seems that no measurement was done, so I suggest to remove.

3. In the present version, there is not enough data on the SEAWATER CHEMISTRY.

Best practices advice to measure at least 2 parameters of the carbonate chemistry in experiments on ocean acidification. A new Table with all carbonate chemistry parameters is needed for both experiment and statistics demonstrating that there was no difference between the replicates.

It is unclear to what experiment the Table 1 is referring to and how the “short” term exposures are included in these values. This can be solved by producing additional figures on the pH/DO changes over the course of the experiments (point 2.2).

4. One striking observation is the important difference in mortality rates between the two experiments (exp1: 0.03 at day 6; exp2: 0.3 at day 5). I wonder if the difference between the two experiments may be related to difference in initial size of the juveniles. It was shown that mortality in juvenile mollusks can be size-dependent, including when exposed to ocean acidification (e.g. Waldbusser et al. 2010). Some information should be provided on initial size in the two experiments and the difference in the control between the two experiments should be discussed. Something that you may see is if there is selection of certain size class under the different treatments (and implications for GR).

5. I also have a more philosophical question. An experiment comparing “constant” vs. “fluctuating” conditions and showing decreased performance in “fluctuating” environment should not be interpreted as a negative effect of the fluctuating environment. The

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fluctuating environment is closer to the natural conditions and should then be considered as the control (Line 97, I suggest to remove “, As a control treatment mimicking typical conditions.” This may not be the real control). What is then shown is a positive effect of the artificial laboratory “constant” conditions.

This is not jeopardizing the experiment or the data. Any difference between the two treatments would be a beautiful “proof of concept” highlighting the importance of including a fluctuating environment in experiment design. This should just be considered in the interpretation of the data.

6. Cumulative mortality is used (Figure 1). This may just hide the real effects (e.g. no effect after 4 days may just be mortality at different times in the different treatments that may be correlated to different growth rates). You should rather calculate a daily mortality rate to identify WHEN (IF?) there is an effect of the treatment.

Another point, it is unclear why you decide to start from 0 after the second exposure for cumulative mortality in the second experiment. Using a daily mortality rate may also clarify this point.

7. The link between increased variability for growth rate and potential for adaptation (e.g. Line 31-33) is a little dangerous. This would only be true if linked to genetic variability and there is no evidence of this in the manuscript (increased “hidden” variability under stress is classic and may just be plasticity). I suggest remove/tone down.

8. Discussion will need major changes after re-analysis and based on the new conclusions.

9. Authors may consider digging into the literature on tidal ecology/physiology. Tidal species are exposed and are adapted to frequent fluctuations. There is useful information on physiological adaptation to change in this literature.

Other comments: Line 25, “higher mortality”, be more specific (e.g. effect size) Line 29, replace “&” by “and” Line 31, replace “pH and oxygen is a crucial factor” by “pH

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and oxygen are crucial factors” Line 40, “nearshore environment”, is this true for all of them? If not, be more specific. Line 51, I suggest to remove “, particularly those that produce calcified structure (e.g., Kroeker et al., 2011).” The link with calcification can be weak as demonstrated by the domination of calcifiers in some upwelling zones (e.g. Kiel fjord, Thomsen et al. 2010).

Hope this help, feel free to contact me if you have any question.

Cheers Sam

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