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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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Dear Dr. Dupont:

We appreciate your comments very much. They led us to rethink our results which hopefully have improved the content and clarity of the manuscript. Following below are responses for each comment.

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,

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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 “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.

Response: As you suggested, we changed the terminology for short-term experiment into Experiment I and long-term experiment into Experiment II throughout the manuscript.

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).

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Response: We incorporated the experimental treatment protocol which was applied to both experiments. We additionally tagged the treatment 1. Low pH (7.5) & Low O2 (40% saturation, 5 mg l-1) =‘ upwelling”; 2. High pH (8.0) & Low O2 = ‘ low oxygen’ and 3. High pH (8.0) & High O2 (100% saturation, 12 mg l-1) as a ‘control’ for better understanding (Lines 102-104). The general experimental procedure for Experiment I was explained in the Experiment I section. The experimental procedure which is applied to only Experiment II was described in the section of Experiment II.

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 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).

Response: We have substituted a new figure for supplementary figure 1, showing the pH of experimental treatments during the experiment as suggested. Unfortunately, although we checked the pH periodically, it was not monitored continuously during this experiment. Measurements of the ambient pH water (nominal pH 8.0) using a UV spectrophotometer (UV-1601 Shimadzu®) at MBARI before (Oct 26, Nov 22, Dec 16 2010) and after the experiment (Mar 30, 2011) were pH 7.99 $\pm$ 0.04, and were very similar to values measured for the high pH & low O2 (pH 8.01 $\pm$ 0.18) treatment and the high pH & high O2 treatment (pH 8.09 $\pm$ 0.18) during the experiment using the portable pH meter. Therefore, we assumed that there was little deviation from pH 8.0 for both in the high pH treatment water in the system and in normal water in the holding tank at MBARI. We added this information in Lines 117- 124. We also included this value (pH 8.0) to the supplementary figure 1. We hope that this figure helps readers to

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understand the experimental conditions.

In addition, we have added some text discussing the dynamic character of pH changes in the Monterey Bay area, based on data from the Monterey Bay Aquarium (Lines 74-76). It is apparent from the Monterey Bay Aquarium seawater intake monitoring data that pH in this area can change dramatically within an hour or less.

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.

Response: Individuals will have different responses to stresses. We agree that death is likely associated with high energy costs that can influence GR. However, we think that although it is worth considering dead and alive separately, it is highly likely that all individuals experienced stress and reacted to varying degrees along a spectrum from mild disruption of metabolic function to severe disruption and death. We measured animals that died immediately after death, and rated their growth as daily increments. Many of the live individuals could also have been very close to death. When analyzed separately, there was no significant relationship between GR or live / dead or interaction with treatment and growth rate (Lines 2229-232). Nevertheless, we coded live / dead status on scatter graphs so that readers may see clearly the growth rates of individuals that survived or died during the experiments.

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).

Response: Mortality was calculated by dividing the number of dead abalone by the number of abalone initially put in each jar (Lines 178-179). We labeled as “proportional mortality” the y-axis of the figure.

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2.5. The method to measure GR (line 160, REM: all line numbers refered to the submited 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).

Response: We assumed linear growth for juvenile abalone because their shell lengths were similar (for both experiments) and the experimental period was short, with small amount of growth in those juveniles. To evaluate the relationship between size and growth, we plotted proportional growth rate versus initial shell size and found no significant slope ($F_{1,149} = 1.80$, $p = 0.1818$), indicating that, at least the range in sizes used here, growth rate is unrelated to size. We added this information in Lines 191-195.

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.)

Response: The reproductive population used by The Abalone Farm for rearing juveniles used in our study was originally collected in Southern California (Lines 86-88). There are no pH data available concerning the conditions under which the abalone obtained from the Abalone farm were reared. We do have temperature data from the farming system. Please see Line 88.

- Line 85, is food 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.

Response: Yes. Food was provided "ad libitum". We observed the remnants of algae wafers every other day (Line 91).

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Line 87, some informationon conditions (temp, pH, etc.) at HMS.

Response: We added information for ambient temperature (Mean ± SD: 12.6 ± 3.4, 11-13.8 °C) and pH (Mean ± SD: 7.79 ± 0.16,Ranges: 7.61-7.98) at HMS (Lines 90-91).

Response:

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"?

Response: pH scale was measured on the total scale and we used pHT in Line 97. All pH meters were regularly calibrated with pH values of Fisher Scientific buffer salt solutions measured by a spectrophotometer (Lines 135-137).

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

Response: Deleted as suggested.

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.

Response: We have added a table with seawater chemistry. To calculate seawater carbonate parameters (pH and TCO2), seawater with Low pH & Low O2 treatment and seawater with High pH & Low O2 treatment were sampled three times during the experimental period. Seawater pH was measured using a spectrophotometric method (Dickson et al. 2007). TCO2 was measured by non-dispersive infrared analysis (LI-COR model 6262), as detailed by Friederich et al. (2002). We added this information in the Methods (Lines 137-141).

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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).

Response: Treatments described in Table 1 were applied to both experiments. Juvenile abalone were held under natural conditions (High pH and High O2) except for the short (∼3H) or long (24H) periods of exposure to upwelling conditions (low pH, low O2) or intermediate conditions (high pH, low O2). We also added figures for pH changes over the course of the two experiments. We hope this resolves the remaining concern.

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).

Response: There was large difference in mortality between the two experiments, even in the control treatment. The initial sizes of the juvenile abalone used did not differ between experiments (Exp. 1 =7.36 $\pm$ 0.76 (Mean $\pm$ SD), Exp. 2 = 7.42 $\pm$ 1.23). We have added information on the size of juvenile abalone for these experiments in the manuscript (Lines 145-146 and 164). We cannot explain the difference in mortality rates, and speculate that extended exposure to low temperature associated with upwelling conditions may also lead to higher mortality. Alternatively, there may be differences in the general health of abalone among batches of juveniles obtained from the Abalone Farm that contributed to differences in mortality between experiments. Obviously, other unknown processes may be involved.

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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 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.

Response: We agree. Our “Control” treatment is not the natural environmental conditions experienced by these animals. We removed the sentence “mimicking typical conditions” but left “control” because we think that to know the effect of exposure to low O2 and/or low pH water, high pH and high oxygen control is needed.

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.

Response: We appreciate your suggestion. However, cumulative mortality and repeated measures ANOVA are widely used to assess the biological effect of stressors on a variety of species. We have also tested daily mortality as suggested and there was not much difference of the results in the statistical test. The cumulative mortality starts from 0 after each exposure because we expected that each exposure will have a separate impact on their mortality. We confirmed that mortality on the day just before the next exposure was not significantly different so there is no more latent impact of first

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exposure. This experimental method and statistical test do not have the problem. We have retained the graph of cumulative mortality rather than substituting with daily mortality because presenting cumulative mortality is more intuitive and easy to understand the effect of treatments.

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.

Response: We toned down the statement by "cryptic phenotypic plasticity may promote acclimatization to prolonged upwelling conditions by a portion of the population. " Lines 30-33.

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

Response: We have changed our discussion following re-analysis.

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.

Response: We added the reference for ecology/physiology of tidal species adapted to fluctuation of pH and oxygen (Lines 49-50). We also added following sentences related to this: The local extremes in several factors (oxygen, pH, and temperature etc.) could be stressful for the population. In particular, juveniles and other sensitive life stages may be highly vulnerable to extremes. Lines 52-54.

Other comments: Line 25, "higher mortality", be more specific (e.g. effect size)

Response: We added "approx. 5-20%" to the higher mortality. See Line 25.

Line 29, replace "&" by "and" Line 31, replace "pH and oxygen is a crucial factor" by

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“pH and oxygen are crucial factors” Line 40, “nearshore environment”, is this true for all of them? If not, be more specific.

Response: We changed them as suggested.

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).

Response: We removed this part from the sentence.

Hope this help, feel free to contact me if you have any question. Cheers Sam Interactive comment on Biogeosciences Discuss., 10, 3559, 2013.

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/10/C2016/2013/bgd-10-C2016-2013-supplement.pdf

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Interactive comment on Biogeosciences Discuss., 10, 3559, 2013.