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Interactive comment on "Effects of CO_2-driven ocean acidification on early life stages of marine medaka (*Oryzias melastigma*)" *by* J. Mu et al.

J. Mu et al.

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Dear Prof.,

Pleased find enclosed our revised MS and the responses to the comments below. We thank very much for your kind and positive words of our manuscript. Both suggestions and criticisms are very valuable and helpful for revising and improving our MS. Below, we have addressed all of the comments and made corresponding revisions in the MS which were marked in red. We hope that you will agree that our MS is now improved and fulfills the requirement for publication in Biogeosciences.

Yours sincerely, Jingli Mu

Comment 1:For the abstract, larval deformity was also mentioned. Thus, it is not only

the calcification of otoliths, which were affected.

Response 1: Clarified in revised MS.

Comment 2:P3, Line 3- "Coccolith" should be "coccolithophores"

Response 2: Revised.

Comment 3:P3, line 17- The otolith is not the only calcified organ of fish. Bones are also calcified.

Response 3: Revised.

Comment 4: P3, line 18-It is not calcite. Rather, it is aragonite.

Response 4: Revised.

Comment 5: P3, line 19-There are more recent studies showing increase otolith calcification but has no effect on fish larval swimming behavior of Atlantic cod and herring. Might consider adding new reports.

Response 5: Added.

Comment6: P3, line 19-Include introduction on how or why embryogenesis and organogenesis will be affected by OA.

Response 6: Added corresponding introduction in MS as suggested (see below). Decades of empirical data suggest that juvenile and adult fish possess sufficient acid-base and osmoregulatory capabilities for the toleration of very high metabolic and ambient CO2 levels (> 2000 μatm) (Murray et al., 2014). Although fish possesses the ability of acid-base balance regulation, its physiological function will certainly decline under such regulation for a long time from the perspective of energetics, especially in the most fragile and sensitive early life stage during its life history. In addition, in early life stages of multiple taxa including fish, elevated CO2 was shown to affect calcification of shells and skeletons due to a drop in the carbonate availability (Riebesell, et al.,

2010).

Comment 7: P4, line 26-Provide references for the CO2 scenarios used.

Response 7: Added.

Comment 8: P5, line 10-30 fish eggs per treatment or replicate?

Response 8: 30 fishes per replicate, and 3 replicates per treatment (a total of 90 fish).

Comment 9: P6, line 7-Were the eggs returned back to the experimental tanks after each observation? Were there any side effects on the eggs because of the method? If eggs were not returned, how many were left until the observation of hatching? and P4, line 14 - How did you calculate the larval deformity rate? Should it be called "proportion of larvae with deformities"?

Response 9: The eggs were returned back to the experimental tanks after each observation. The sampling was gentle and the observation was accomplished within a couple of minutes for each replicate, in which process the side effect on the eggswas negligible in such short time compared to their exposure duration. As for the larval deformity rate, on day 21, thirty larvae (10 larvae per replicate) from each CO2 treatment were randomly selected and photographed for deformity analyses. The deformity rate were calculated based on the proportions of abnormal larvae numbers out of 10 eggs in per replicate. Larval deformity rate is a common phrase which is also generally used in other literature.

Comment 10: P6, line 18- How was the correct identification of the position of the otoliths ensured during extraction? Were adhering tissue materials removed from the otoliths?

Response 10: Body transparent is one of the advantages of marine medaka recommended as a model fish, and we can see the position of the otoliths under microscope (see the figure below). Before the measurement, adhering tissue materials has been removed from otolith to ensure the accuracy of the measurement. (Supplement. Fig. 1

The position of otolith in marine medaka larvae)

Comment 11: P6, line 18- year of Franke and Clemmesen?

Response 11:Revised.

Comment 12: P7, line 5- How was the rate calculated for deformities?

Response 12:Please see the Response 9.

Comment 13: P7, line 5- So far, no hypothesis or principle on why comparison between the left and right side otoliths was made.

Response 13: Any substantial change inthe size, shape, or symmetry of otoliths could have serious implications for individual performance and survival (Gagliano et al., 2008, Dispersal without errors: symmetrical ears tune into the right frequency for survival. Proc. R. Soc. B., 275: 527-534.). Munday et al. (2011) found that mean area of otoliths in the pH 7.6 treatment was larger than that of control for left otoliths, but not right otoliths. Therefore, it is very necessary to compare the difference between the left and right otoliths. (Munday et al., 20011, Effect of ocean acidification on otolith development in larvae of a tropical marine fish, Biogeosciences 8, 1631-1641.)

Comment 14: P7, line 9- Change "proceed" with "used".

Response 14:Changed.

Comment 15: P7, Results - How was the size (standard length) of the fish larvae affected by OA? Otolith size is influenced by fish size and growth rate. Thus, it must be taken into account when analyzing the effects of OA on otolith growth.

Response 15: Thanks for your suggest. In order to ensure the synchronization of the experimental eggs, all eggs were collected within 3–5 h after spawning and screened with a stereoscope to ensure normal fertilization and development of each egg. As the reviewer mentioned, the size of fish larvae is an important parameter for assessing the effects of OA to otoliths. It is a pity that the size of fish larvae was not measured in this

study. However, we would like to adopt the reviewer's suggestions and take the size of fish larvae into accountin our future researches.

Comment 16: P7, line 9- 'Seawater chemical parameters' Transfer to Methods section.

Response 16: For the study of OA effects on fishes, we considered that the seawater chemical parameters were an important part of results to ensure the stability of pH control system, therefore we prefer to retain 'Seawater chemical parameters' in Results section.

Comment 17: P7, line 23-add F statistics and sample sizes. Is the p-value for all the three parameters?

Response 17:Added.

Comment 18: P8, line 21- "decrease" should be "increase".

Response 18:Revised.

Comment 19: P9, line 24- Is this from Baumann et al., 2012 also? Is it negatively or positively related? In the next sentence, it is stated that survival and length significantly decreased at 1000uatm, which means negatively correlated.

Response19:Yes it is from Baumann et al., 2012. We clarified the sentence as below: Baumann et al. (2012) reported a 74% reduction in survival of embryos and young larvae of inland silverside, Menidiaberyllina, native to estuaries of the US Atlantic coast, when maintained at 1100 ppm pCO2 compared to those held at 410 ppm pCO2.

Comment 20: P10, line 3- There was no mention of the procedure that analysis of deformities was done in the embryos. Thus, on the basis of deformities observed in newly-hatched larvae alone, one cannot make comparison of the vulnerability between embryo and larvae. Also, heart rate was not monitored in the larvae.

Response 20:We added the procedure regarding the analysis of deformity in revised MS.

Comment 21: P10, line 3-What is the role of Cadmium ions in this context?

Response 21: Sorry, "Cadmium" should be "calcium".

Comment 22: P10, line 9- These are not parts of the otolith. These are three types or pairs of otoliths.

Response 22: Revised.

Comment 23: P10, line 9-Its formation starts during...

Response 23: Revised.

Comment 24: P10, line 10-important? Maybe you meant alteration of otolith size or shape has implications on physical performance... Not "important for..."

Response 24: Revised and further described the procedure of the analysis of deformity. Revisions in MS is as below: Therefore, any substantial change to the size, shape, or symmetry of otoliths could have serious implications for individual performance and survival (Munday et al., 2008, 2011).

Comment 25: P10, line 10-should be "calcium carbonate structure"

Response 25: Revised.

Comment 26: P10, line 14- There is a natural fluctuation of sizes between the left and right otoliths, without preference to any side. This applies on the individual level. If you compare the two sides by combining all the data from each side from all the individuals, the inherent natural fluctuation between the sides is masked. A comparison of the magnitude of absolute differences between the left and right sides (Fluctuating Asymmetry) can be made among the treatments.

Response 26:The difference between the right and left side was compared only under the same CO2 treatment, but did not among three CO2 treatments. There was no systematic pattern of deviation from the normal fluctuating asymmetry, and these find-

ings support the earlier report by Munday et al. (2011) on the maintenance of otolith symmetry.

Comment 27: P10, line 15-Kindly double check the analysis because the standard deviations in the Figure 5B overlaps among the treatments. Also, check the influence of fish sizes on the sizes of the otoliths.

Response 27: In pH 7.6 treatment, the average area of left and right sides was significantly smaller than otoliths from control treatment ($F_{1, 128} = 8.8$, $p = 0.013$) (Fig. 6B).

Comment 28: P10, line 18- Perhaps, the deformities observed have more significant implications on the survival of the larvae compared to the otolith calcification. Please add references on hypercalcified larval fish otoliths with corresponding no negative impacts on the swimming behavior. There are other studies showing that even absence of 1 or more otoliths, behavior of fish larvae was not affected (e.g. zebrafish).

Response 28: The relevant reference was added as recommended in revised MS.

Comment 29: P10, line 20- This discussion on acid-base regulation ability of the fish larvae is not clearly supported by Figure 5B of the paper.

Response29: We have made corresponding revisions in manuscript as recommended.

Comment 30: P11, line11- There seems to be clear impact of elevated pCO2 on the formation of deformities on the larvae. However, this was not well discussed in the discussion. Instead, there was a bias towards expounding the issue of otolith calcification. Less emphasis was also placed on the non-significant effects on hatch rate, hatching time and heart rates. These data could point to the relative resilience of the species towards elevated pCO2 scenarios. And thus, could be further discussed in the paper.

Response 30:Revised as below: In conclusion, this study demonstrates that, even under projected near-future pCO2 levels, the early life stages of marine medaka exhibited a dramatic increase of their larval development deformity and otolith calcification while

their survival was not affected. Importantly, the observed CO2-induced abnormal development of larvae might have predictably negative consequences on the recruitment of fish population, the effects of whichon later life history and the phenotype of subsequent generations of ocean acidification on marine fish should be concerned.

Comment 31: P19 The types of deformities can be further described in the paper. If possible, provide higher magnification picture to show the deformities.

Response 31: Further descriptions of deformities were added and higher magnification pictureshowing the deformities were also provided in MS.

Comment 32: P20- Asterisk is not very informative. Is pH 7.6 significantly different from pH 7.2 as well?explanation for panels A and B

Response 32: Explained. Asterisk indicates that the values of pH 7.6 differed significantly from the control (pH 8.2).

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/12/C886/2015/bgd-12-C886-2015-supplement.pdf

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