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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. General Comments: This study investigated the effects of elevated sea-

water pCO2on the embryogenesis and organogenesis of marine medaka (Oryzias melastigma). Newly fertilized embryos were exposed to control (pH 8.2) and reduced (pH 7.6 7.2) pH levels for 21 days. The experimenters found no significant differences in hatching time, hatching success, and larval heart rate between pH treatments. However, the pH 7.2 treatment was found to cause significantly more developmental abnormalities than the control; including spinal deformities, craniofacial deformities, stretched heart and pericardial edema. In addition, the researchers found slight differences in otolith development. The average areas of the left and right sagittae were significantly smaller in the pH 7.6 treatment than the control. Such an effect was absent in the pH 7.2 treatment.The study provides needed data on the effects of elevated pCO2 on fish early life stages from a marine species. Such studies are valuable given the current uncertainty surrounding the potential effect of ocean acidification on fish early life stages, a topic that's suitable for Biogeosciences. However, I cannot recommend this manuscript for publication until inaccuracies in the description of other studies regarding ocean acidification and marine fish early life stages are corrected and uncertainties in their methodology clarified. Major Concern 1: Although it may be unintentional, the authors misstate some findings in previously published literature

Response 1: Thanks for your recognition and positive comments of our work. We realize the inaccuracies in the description of other studies regarding ocean acidification and marine fish early life stages and corrected now in the MS. We also clarified some findings in previously published literature in the MS as recommended.

Comment 2: Example 1 (P9, L222) the authors write "Based on our results, the tolerance of marine medaka to increased CO2 level is possibly stronger than fish such as red seabream and Japanese whiting. The reason is likely attributed to different life history and living habit of fishes. Marine medaka, which lives in estuary and adapts to differently environmental salinities, possess some ability to adjust a range of pH fluctuation, whileoffshore coral reef fish, red seabream, has a strict requirement of environmental factorssuch as salinity and DO for growth and production. Therefore, red

seabream has a bad adaption to CO2-driven pH fluctuation." The authors are referring to the findingsin Kikkawa et al. 2004 where red seabream Pagrus major were exposed to pH levelsof 6.2 and 5.8. These levels are not relevant in the context of future ocean acidification. Thus, suggesting red seabream have a substantially lower tolerance to CO2 than medaka is inaccurate, the studies are not comparable due to significant differences inmethodology.

Response 2: We agree with the reviewer's comments and made corresponding revisions in the manuscript. Below are our clarifications: In our experiments, the duration of embryonic stage, egg survival and embryonic heart rate of marine medaka were unaffected by acidification water with pH 7.6 and pH 7.2. There was a slight increase in embryonic duration of eggs, but the size effect was not different among the three pH treatments. Overall, these results suggest that the egg stage of marine medaka is relatively tolerant to elevated CO2 and low pH level, which were consistent with the results reported by other studies on a diverse set of marine fishes. For instance, Munday et al., (2009) found the survival to hatch of orange clownfish (Amphiprion percula) from the Great Barrier Reef, Australia, to be nonresponsive to pCO2 levels to 1020 ppm (pH 7.8). Similarly, Franke and Clemmesen (2012) found no significant effect of elevated pCO2 levels from 460 to 4635 ppm (corresponding to pH 8.08 pH 7.05) on survival to hatch of Atlantic herring from the western Baltic Sea. In the study of Frommel et al. (2013), the survival of embryos of Atlantic cod from the Bornholm Basin of the western Baltic Sea was not altered at pCO2 levels up to 4000 ppm (pH 7.2). Hurst et al. (2013) also reported no effect on embryo survival of walleye pollock (Theragra chalcogramma), common in the temperate eastern North Pacific, at pCO2 levels up to 1933 ppm (pH 7.4). In other cases, however, a strong effect of CO2 was observed evident in the embryo survival of summer flounder (Paralichthys dentatus), an ecologically and economically important flatfish of the inshore and nearshore waters of the Mid-Atlantic Bight (Chambers et al., 2013). The relative survival of summer flounder embryos was reduced to 48

Comment 3: Example 2 (P10 L230) the authors write that "Inland silverside is also common in estuary;however, the survival and length of larvae are positively related with CO2 concentrationwhich is possibly associated with its life history". Presumably, the authorsare referring to the findings of Baumann et al. 2012 that actually showed the opposite effect, survival and growth were negatively correlated with increasing CO2 concentrations.

Response 3: Corrected. The corresponding revisions in MS are as below: All of these studies varied in the number of parents used, the time lapse between egg fertilization and the initiation of the CO2 treatment, and in how and when the survival was scored. For example, the CO2 treatments of inland silverside by Baumann et al. (2012) began at approximately 24 h post-fertilization, and the survival was scored at approximately 1 week post-hatching. The different approaches used among previous studies may preclude a fair cross-study comparison (Chambers et al., 2014), however, the overall presence of effect of elevated CO2 environments on embryo survival is in contrast to the findings here. The habitats occupied by a species, particularly its ELS, may play a role in their sensitivities whose ELS are found in estuarine (marine medaka) or inner shelf (summer flounder) habitats, both with relatively high ambient CO2 levels, exhibit different sensitivity to experimentally elevated-CO2 levels which is counter to expectations and requires further attention.

Comment 4: Example 3 (P10 L 232) the authors write "In addition, research on inland silversidefound that survival and body length of larvae significantly decreased compared to thecontrol group after exposure to 1000atm CO2 for 7 days, while those of embryos werenot affected, indicating more sensitivity of larvae to CO2 than embryos. The reasonswere attributed to the self-protection of fertilized embryos and their less dependenceon external environment (Baumann et al., 2012)." This statement is confused andinaccurate. Baumann et al. 2012 found the embryonic stage to be more sensitivethan the larval stage, and never concluded that 'self-protection' of fertilized embryosincreased their CO2 tolerance.

Response 4: According to Baumann et al. (2012), when compared with present-day CO2 levels (400 ppm), exposure of Inland Silverside (Menidia beryllina) embryos to 1000 ppm until one week post-hatch reduced average survival and length by 74An unexpected result of our study was that elevated levels of CO2 affected larval development abnormalities, and the average deformity rate of marine medaka larvae (approximately one-week post-hatch) increased significantly ($p < 0.001$) by 16

Comment 5: Example 4 The authors make conclusions regarding their results based on their inaccurate understanding of previous studies. For example, on P10 L237 the authors write “Interestingly, our results seemed to support the above conclusion as heart rate, hatchingrate and hatching time of marine medaka embryos were not significantly affected while obvious deformities were observed in newly-hatched larvae, suggesting the latterwas more liable to be influenced by OA.” Presumably the authors are suggesting their results that embryos appear unaffected by CO2 but larvae show deformities, corroboratethe findings in Baumann et al. 2012. Again, Baumann et al. 2012 concluded thatembryos were most sensitive to CO2.

Response 5: Based on the findings of Baumann et al. (2012), we have made revisions in manuscript (as shown in response to comment 4) .

Comment 6 : Major Concern 2: The methodology employed for the developmental toxicity may need further clarification. How was deformity rate calculated? Is it simply the proportionof larvae, which demonstrated one of the mentioned developmental deformities? Theauthors sampled both embryos and larvae for analysis. Does the calculated deformityrate include both? This is unclear. In addition, were embryo or larval samples replacedafter analysis? If so, with a rather small sample size, how did the authors take intoaccount the possibility of resampling? I worry about the conclusiveness of their toxicologyresults given the uncertainties in their methodology. Also, presumably the authorsmaintained survival data during this experiment. Such data would be extremely usefulfor other investigators and I wonder why it was not presented.

Response 6: The detailed protocols for the developmental toxicity were described in the revised manuscript according to the reviewer’s suggestion (see section 2.4). For the sample size, three replicates produced a total of 90 eggs in each pH groups during the embryonic stage, and three replicates produced a total of 66 75 newly hatched larvae in each CO2 treatment level. The data of survival larvae were added in revised manuscript as recommended. The methodology employed for the developmental toxicity were shown as follows: The numbers of embryos surviving to hatching were counted based on daily inspection of the embryos in each treatment. Hatching rate data were summed and converted to proportions of survival numbers out of 30 eggs in per replicate. After 8 days post fertilization, and 3 days before expected hatching, embryos were inspected at least twice a day to record hatching numbers. Estimates of heart rate were completed by counting the number of heart beats over a 30 s period (n = 10) at day 8. The time when $\geq$ 50 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. Survival rate of larvae was the obtained proportions through dividingthe larvae numbers remaining at termination of the experiment by the larvae numbers initially newly hatched in per replicate.

Comment 7 :Specific Comments: (1) P3 L40 I believe it is premature to state that OA will havesevere consequences for marine organisms, for you reasons described in the second half of this sentence.

Response 7: Revised as below: One alarming consequence is a rapid change in seawater chemistry and decrease of ocean pH, which could have great impacts on marine ecosystems, and pose a threat to marine life (Frommel et al., 2013a; Kerr, 2010). Elevated CO2 concentrations can disturb the acid-base regulation, blood circulation, respiration, as well as the nervous system of marine organisms, leading to long-term effects such as reduced growth rates and reproduction (Frommel et al., 2013a). Other directs of ocean acidification have been found in the alteration of behavior (Dixson et

al., 2010; Munday et al., 2009a), development (Frommel et al., 2012b), RNA/DNA ratio (Franke and Clemmesen, 2011), and otholiths (Checkley et al., 2009; Maneja et al., 2013; Munday et al., 2011b) of marine fish larvae.

Comment 8:P3 L50 This sentence is awkward, authors should restructure it to increase clarity.

Response 8: Reworded.

Comment 9: P3 L54 The authors state fish physiology will “certainly decline” during acid/base regulation induced by ocean acidification. This statement is too strong, the current literature demonstrates a variety of response, many of which are neutral or minimal.

Response 9: Revised in the MS as below: However, the emerging picture remains intriguingly complex. While the majority of responses to high CO2 appear to be negative (Branch et al., 2013) with highest sensitivities observed during the early life stages and in calcifying invertebrates such as corals, bivalves, pteropods, and echinoderms, there arealso substantial evidences for non-linear, neutral, or even positive reaction to increasing CO2 conditions (Hurst et al., 2013; Munday et al., 2011b; Murray et al., 2014).

Comment 10: P8 L194 The sentence that starts as “A number of studies found: ” needs additionaland more appropriate citations.

Response 10: Revised as recommended.

Comment 11: P9 L202 This sentence is in contradiction tothe results presented in Figure 3, which shows deformity rate increased, rather than decreased, under pH 7.2.

Response 11: Sorry for the confusion and we have clarified this sentence in revised MS (see below). An unexpected result of our study was that elevated levels of CO2 affected larval development abnormalities, and the average deformity rate of marine medaka larvae (approximately one-week post-hatch) increased significantly by 16

Comment 12: P10 L255 A more detailed discussion on why elevated CO2 decreased otolith area rather than increase (as seen in many other studies) isneeded. The appearance of this effect at pH 7.6 and not 7.2 also requires further explanation.

Response 12: We have made corresponding revisions in manuscript as required.

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