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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.

Anonymous Referee #1

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General Comments: This study investigated the effects of elevated seawater pCO2 on 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 uncer-

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tainty 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.

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, while offshore coral reef fish, red seabream, has a strict requirement of environmental factors such 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 findings in Kikkawa et al. 2004 where red seabream Pagrus major were exposed to pH levels of 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 in methodology.

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 concentration which is possibly associated with its life history". Presumably, the authors are 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.

Example 3 (P10 L 232) the authors write "In addition, research on inland silverside found that survival and body length of larvae significantly decreased compared to the control group after exposure to 1000 μatm CO2 for 7 days, while those of embryos were

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not affected, indicating more sensitivity of larvae to CO_2 than embryos. The reasons were attributed to the self-protection of fertilized embryos and their less dependence on external environment (Baumann et al., 2012)." This statement is confused and inaccurate. Baumann et al. 2012 found the embryonic stage to be more sensitive than the larval stage, and never concluded that 'self-protection' of fertilized embryos increased their CO_2 tolerance.

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, hatching rate and hatching time of marine medaka embryos were not significantly affected while obvious deformities were observed in newly-hatched larvae, suggesting the latter was more liable to be influenced by OA." Presumably the authors are suggesting their results that embryos appear unaffected by CO_2 but larvae show deformities, corroborate the findings in Baumann et al. 2012. Again, Baumann et al. 2012 concluded that embryos were most sensitive to CO_2.

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

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

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(2) P3 L50 This sentence is awkward, authors should restructure it to increase clarity

(3) 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.

(4) P8 L194 The sentence that starts as “A number of studies found. . .” needs additional and more appropriate citations. (5) P9 L202 This sentence is in contradiction to the results presented in Figure 3, which shows deformity rate increased, rather than decreased, under pH 7.2. (6) P10 L255 A more detailed discussion on why elevated CO2 decreased otolith area rather than increase (as seen in many other studies) is needed. The appearance of this effect at pH 7.6 and not 7.2 also requires further explanation.

Literature Cited Baumann H, Talmage SC, Gobler CJ (2012) Reduced early life growth and survival in a fish in direct response to increased carbon dioxide. Nature Climate Change 2:38-41 Kikkawa T, Kita J, Ishimatsu A (2004) Comparison of the lethal effect of CO 2 and acidification on red sea bream (Pagrus major) during the early developmental stages. Marine Pollution Bulletin 48:108-110

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