

## ***Interactive comment on “Is the perceived resiliency of fish larvae to ocean acidification masking more subtle effects?” by E. C. Pope et al.***

**Anonymous Referee #1**

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Journal: BG Title: Is the perceived resiliency of fish larvae to ocean acidification masking more subtle effects? Author(s): E. C. Pope et al. MS No.: bg-2013-504 MS Type: Research Article

Overall review:

The manuscript is well written and, although the design of the experiment is somewhat flawed, the experiments seem to have been performed with care. The title of the manuscript is misleading and I would propose to change it to something more informative of the content such as its short title. The manuscript would improve greatly from a more thorough and critical interpretation and discussion of the results and less review of available literature. I would suggest rewriting the discussion (and the abstract) with a focus on the available data, comparing larval and juvenile development, growth,

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mortality, food consumption and metabolic rates. Care should be taken when making claims for novelty and sweeping statements on the future of *D. labrax* with the available data (see specific issues).

Specific issues:

1. Animals: The eggs used for this study came from aquaculture? *D. labrax* is a species intensively used in aquaculture and therefore these animals may differ in their response to CO<sub>2</sub> compared to wild populations. For a discussion on this see Parker et al. Mar Biol 2011 and Donelson et al. Nat Clim Change 2011. Additionally, how many females and males were used to spawn the eggs? It is not enough to use large numbers of eggs. Genetic diversity is needed to make broad statements about an entire species.

2. CO<sub>2</sub> manipulation: Firstly, as can be seen in the diagram showing the set-up of the experiment (Fig 1), the “replicates” are pseudo-replicates and are not independent of each other. CO<sub>2</sub> was manipulated in a header tank that was then distributed to three tanks in which the larvae were reared. “any treatment must be replicated at the level at which the treatment is applied” (See Guide to best practices for OA research: Experimental design). Therefore, the data must be interpreted with more caution, especially since only two levels of CO<sub>2</sub> were used. Further, in the diagram it looks as though the tanks in the treatment level were blocked together? This would also be a design flaw. Secondly, DIC was not measured. Instead, only alkalinity and pH measurements were used to calculate the carbon system. To insure reliability of quality measurements, more information on the pH probe and calibration should be given. See Guide to best practices for ocean CO<sub>2</sub> measurements . How do the temperature and CO<sub>2</sub> levels compare to the normal range this species encounters in the wild? How do the levels compare with those the parental animals were used to from aquaculture? Animals kept in large numbers in aquaculture may already be used to high CO<sub>2</sub> levels and normal holding temperatures may be different to those found in the field (i.e. fish may be kept at higher T in order to achieve faster growth rates).

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3. Mortality: When was mortality measured? Only on day 46? Does it make sense to calculate a daily mortality rate over such a long period? Do we assume mortality to be linear over the entire course of larval development? Not really. We would expect different stages of larval development to be more vulnerable than others, as the authors themselves stated with weaning to dry food. The same with growth. Growth is not linear over the course of larval development. For a nice review and summary on the topic (including *D. labrax*) see Ed Houde's paper in Fishery Bulletin 1989.

4. Respirometry: The fish used for this part of the experiment have a HUGE size range (58 – 649 mg WW). I am not sure that with this large range in size it is enough to use a metabolic scaling coefficient of 0.8 to remove size as an effect. I would like to see a graph plotting oxygen consumption over size and I think WW should be included as a random factor in the analysis of O<sub>2</sub> consumption, T and CO<sub>2</sub>.

5. Size/growth/development: Juveniles have a significantly higher wet weight at higher T and CO<sub>2</sub>. What about the dry weight at this stage? When do the larvae metamorphose to juveniles and what distinguishes the larval from the juvenile phase? Did this differ between treatments? The authors observe developmental differences between the larvae/juveniles raised under different temperatures. Then how can metabolic rates be compared between the treatments, if the fish differ in size and developmental stage between the treatments?

6. Figures: The manuscript would benefit from condensing information and focusing on the essentials. See details below.

Intro: p. 17045 lines 17-25: old news. remove. p. 17046 line 16: inappropriate reference. p. 17047 lines 7-11: such claims at novelty should be removed.

Material and Methods 2.3 animals: p. 17048 line 25-26: how much food was given to the larvae? What are the units given? rotifer/artemia individuals per ml? Is this ad libitum or was the density of food adjusted to the density of fish larvae in the tanks? 2.4 sampling: too detailed. Remove discussion on volumetric sampling (lines 6-10) 2.6

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respirometry: too detailed. p. 17051 remove lines 4-6 on and condense lines 15 – 28.

Results: 3.1 main incubation: since water parameters are summarized in table 1, these details do not need to be repeated in text form in the results section. Remove lines 2-13 on p. 17054. As discussed above, I don't think it makes sense to calculate a daily mortality rate over 42 days with only 2 data points, start and finish. The authors should calculate an overall mortality and do statistics on this number instead of on Z. What does the weaning trial tell us?

Discussion: In light of the given data, I do not think the first sentence is appropriate. Looking at Fig. 2a there doesn't seem to be a significant difference in final numbers between larvae raised under ambient and acidified conditions at elevated temperatures. I would like to see the statistics where CO<sub>2</sub> and T leads to higher daily survival. Furthermore, when looking at Fig. 2g we see that at day 28 the larvae have about double the amount of food in their gut under high CO<sub>2</sub> and high T than ambient CO<sub>2</sub> and high T. Although this is only one snapshot, this leads to the assumption that *D. labrax* larvae have to ingest double the amount of food to maintain their body weight at this developmental stage. How would the results have differed, had the larvae been fed less than ad libitum? Would they have been significantly smaller than the control animals? And if this could be the case, how are the results applicable to the field, where larvae often encounter food shortages? Might the experimental design be the actual factor "masking" effects of CO<sub>2</sub>? And how can the lower ingestion of food on day 42 in high CO<sub>2</sub>, high T larvae be explained? The authors should be careful in misinterpreting non-significant results. For a nice review on the pitfalls of non-significant results and experimental design, see online presentations by Jon Havenhand. (ex. [http://www.imr.no/filarkiv/2011/10/havenhand\\_oa\\_seminar\\_tromso\\_2011.pdf](http://www.imr.no/filarkiv/2011/10/havenhand_oa_seminar_tromso_2011.pdf) and epoca website)

p.17058 line 13: . . .observed daily mortalities substantially lower than those observed in a similar study. . . were daily mortality rates really observed, or do we only have the final number of larvae on day 46? Line 21: Frommel et al did not see increased

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survival under 1800 uatm CO<sub>2</sub>. Looking at the range in survival between the replicates and the treatments, the results are inconclusive. The authors should be careful not to misinterpret results. p. 17059 line 7: . . .metabolic rate and aerobic scope were highly dependent upon size. . . I would like to see the authors' data better analyzed taking size into account. Line 22-23: . . .juvenile sea bass at 19C and 750uatm CO<sub>2</sub> were sign. heavier. . . did these larvae also eat more? Could this rapid growth come at a cost as seen in Wood 2008 or Frommel 2011? p. 17060 line 5: . . .no observed effect of pCO<sub>2</sub> or T on feeding. . . ? What about Fig 2g? line 8: if larvae were an average of 72 dd older at 19C than those at 17C then we are comparing apples with bananas. See papers by Dupont on effects of relative age of urchin larvae and CO<sub>2</sub>.

Figures: Figure 2 is difficult to read due to small size and too many panels. Fig 2 a) and b) are essentially the same. Remove 2b). Fig 2 c), d) and e) are also essentially the same. Keep 2d) and remove the others, they do not add any information. Fig 3. What does this graph show us, other than no sign. diff? This figure can be removed. Fig 4 a) what about dry weight? I would also like to see the aerobic scope plotted over wet weight as well as developmental stage.

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