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

E. C. Pope et al.

e.c.pope@swansea.ac.uk

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Dear Dr. Gattuso,

We have read through the interactive comments from anonymous referees 1 and 2 and made substantial changes to the manuscript. We would like to take this opportunity to thank both referees for their insightful comments, we feel they have contributed to a much stronger manuscript.

The two referees both draw attention to the emphasis on a negative interpretation of the results. This is a fair comment and on re-reading the manuscript, we agree with the referees and have made substantial revisions, including changing the title. The revised manuscript is attached as the supplement to this response.

Anonymous referee #1 Specific issues: 1. Animals: The referee correctly identifies that D. labrax is bred intensively in aquaculture and therefore aquaculture animals may differ in response to CO2 compared to wild populations. ACTION: Text has been added to the Discussion stating that the animals came from aquaculture, and the possible consequences are also explored. The suppliers have been unable to state how many males and females were used to produce the fertilised eggs, but it was definitely a mixed spawning (multiple males and females); this is also stated clearly in the text.

2. CO2 manipulation: Both referees #1 and #2 have queried this area of the manuscript. Upon further work, it has become clear that the buffers used to calibrate the CO2 probe meant that we were using pHNBS for our measurements, not pHT, which has significantly affected our calculated pCO2 values. ACTION: The manuscript has been rewritten giving the new pCO2 values throughout as "ambient" and "1,000 μatm". There is additional text on the calculated pCO2 values in both the Results and Discussion.

On the issue of pseudoreplication, this is undoubtedly important but we feel we have explicitly drawn attention to the design of this study and, as noted by anonymous referee #2, it would be effectively impossible to do the study any other way. The issue of pseudoreplication can be very complicated in these kind of trials as there is always the next level of pseudoreplication to consider (e.g. ideally, we would have used independent RASs). We feel that we coped with the logistics of this type of study well and the blocked design was not "flawed" as referee 1 states. We do not see how another design, given the available equipment, would have been an improvement and would like to emphasise that the daily pH and temperature readings were taken from each experimental tank. ACTION: none required

3. Mortality: both referees #1 and #2 pointed out that calculating daily mortality over 42 d assumes that the rate is linear, which it likely is not. It is important to note, however, that this computation of daily mortality, whilst assuming a linear rate, does take into account the regular sampling events and provides a more accurate estimation

of mortality than simply looking at the final numbers. ACTION: We feel that this is valuable extra information and should stay in the manuscript, although the limitation of the technique are now explored in the Discussion. To address the point that growth is also not linear over development, we have replaced Fig 2c with a Fig. 3b showing specific growth, μ, between each sampling period.

4. Respirometry: the juvenile fish did indeed show a substantial size range and a graph plotting oxygen consumption vs. wet weight (WW; Fig. R1 in this reply-to-referees document) shows a clear relationship. ACTION: The calculations have been revised for the respirometry and values are now normalised to a 300 mg (mid-range) fish using a metabolic scaling coefficient of 0.8 (as explained in the original manuscript). As Figure R2 shows, this normalisation successfully removes the relationship between MO2 and WW. We have provided Figs. R1 and R2 in this reply-to referees document (see below) but do not believe they need to be included in the article or Supplementary Information.

5. Size/growth/development: Dry weight was used for larvae because wet weight is so unreliable for small animals. We did not record dry weight for juvenile animals because they were much larger. As for development, we did not observe developmental differences between treatments by d42, although as mentioned in the original manuscript, there was evidence of differences starting to appear (C:N ratio, eye diameter) by d42. These differences were related to temperature, not pCO2, and so comparisons between fish raised at the same temperatures but different pCO2s are still valid. ACTION: none required.

6. Suggested revision to Introduction: p. 17045 lines 17-25: ACTION: This text has been revised but the reference to a deficiency in studies in fish, as demonstrated by Kroeker et al. (2013), has been left in. P17046 line 16: this reference has been changed. P17047 lines 7-11: Claims of novelty have been removed.

Materials and Methods 2.3 Animals p.17048 lines 25-26: Animals were fed ad libitum

and the units were individual rotifers/Artemia mL-1. ACTION: The text now states this. 2.4 p.17049 lines 6-10: Text concerning volumetric sampling has been moved to the Discussion section on mortality. 2.6 Respirometry p. 17051 lines 4-6 have been removed and lines 15-28 have been condensed to make the method more clear.

Suggest revision to Results:3.1 p. 17054. ACTION: lines 2-13 have been removed. As discussed above, we feel that daily mortality is a more useful statistic than overall mortality because the model used to calculate it includes the animals removed during sampling.

Discussion: Referee #1 recommends removing the first sentence but referee #2 says it is important to leave it in. ACTION: We have decided to leave this sentence in with the caveat “appear to be” added and “showing increased survival” changed to “showing decreased mortality”. The Discussion text has also been altered so that the feeding regime of the animals is covered in more detail, especially in respect to the finding of this study. p.17058 line 13: “observed” has been changed to “calculated”. P. 17058 line 21: The text has been revised. Suggest revision to p. 17059 line 7: The scaling exponent of 0.8 has been effective in the analysis. In addition, it matches closely the values calculated by Killen et al. (2007), who measured metabolic rate across the entire ontogeny (weight changes of 6 orders of magnitude) of 3 teleosts, concluding the scaling exponent for Standard Metabolic Rate in most teleosts is 0.80-0.85. ACTION: The calculations have been revised for the respirometry and values are now normalised to a 300 mg (mid-range) fish using a metabolic scaling coefficient of 0.8 Suggest revision to p. 17059 lines 22-23: The larger fish did not eat significantly more, although these data were highly variable. ACTION: Text has been added to suggest that the heavier larvae may indeed have been eating more but that the experimental design was unable to detect this. Suggest revision to p. 17060 line 5 – There was no significant (detectable) difference of pCO2 on feeding. ACTION: The differences in Fig. 2g are not significant because of the variation, although new text has been added to discuss this. Larvae at 19°C were indeed, on average, 72 degree days older than those at 17°C. The Discus-

sion specifically draws attention to this and to extend the metaphor used by referee #1, we are actually comparing two groups of apples grown under different conditions and two groups of bananas grown under those same conditions, which is perfectly valid.

Suggest revision to Figures: ACTION: The data illustrated in Fig. 2 have now been divided into Figs. 2-4. As recommended, we have removed the original Fig. 2c and Fig. 2d. Fig. 2b (daily mortality) has been kept for the reasons stated earlier. The original Fig. 2c (μ) has been replaced with Fig. 3b, which provides μ at different time points, as explained earlier.

Anonymous referee #2

Specific comments:

Suggest revision to Title - ACTION: the title has been changed as requested.

Abstract: Suggest revision to Line 14- ACTION: “However” has been removed.

Suggest revision to 17046, line 3- ACTION: “impact” has been changed to “influence”.

Suggest revision to 17047, lines 3-5- ACTION: This text has been placed in the main text as suggested.

Suggest revision to 17047, line 24: The referee is absolutely correct. ACTION: As stated earlier, pCO2 values have been recalculated and pHNBS now used throughout the document.

Suggest revision to 17048, line 5- ACTION: Text has been changed as requested.

Suggest revision to 17049 and elsewhere - As stated earlier, daily mortality is a useful statistic as it accounts for losses from regular sampling. ACTION: as in response to ref#1; point 3.

Suggest revision to 17053- ACTION: Text for statistical analysis has been changed to give more information on statistical methods.

Suggest revision to 17054 - Samples for TA, silicate and phosphate analysis were actually taken from the header tanks. pH and temperature measurements were taken in each experimental tank. ACTION: Methods have been amended to make this clear.

Suggest revision to 17054 - ACTION: consistent with the request made by referees #1, this paragraph has been removed.

Suggest revision to 17054 – All tanks contained larvae at d42 but the sampling at this time fully depleted one tank (tank 7). ACTION: Text has been amended to make this clearer.

Suggest revision to 17056- ACTION: Results have been changed so that they now only report the factorial aerobic scope. The difference in FAS only occurs at 19°C. The text has been changed to make this clearer.

FIGURE LEGENDS

Fig. R1. Oxygen consumption vs. wet weight for juvenile (d67-69 post-hatch) D. labrax. Regressions are power curves (y = axˆb) using an exponent value of 0.8. RMR = routine metabolic rate, MMR = maximal metabolic rate.

Fig. R2. Normalised (300 mg individual, scaling exponent of 0.8) oxygen consumption of juvenile (d67-69 post-hatch) D. labrax vs. wet weight. RMR = routine metabolic rate, MMR = maximal metabolic rate. Linear regressions of both normalised RMR ($F_{1,58}$ = 0.03391, p=0.8545) and normalised RMR ($F_{1,58}$ = 0.09751, p=0.7560) do not have slopes significantly different from 0.

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/10/C8451/2014/bgd-10-C8451-2014-supplement.pdf

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Interactive comment on Biogeosciences Discuss., 10, 17043, 2013.

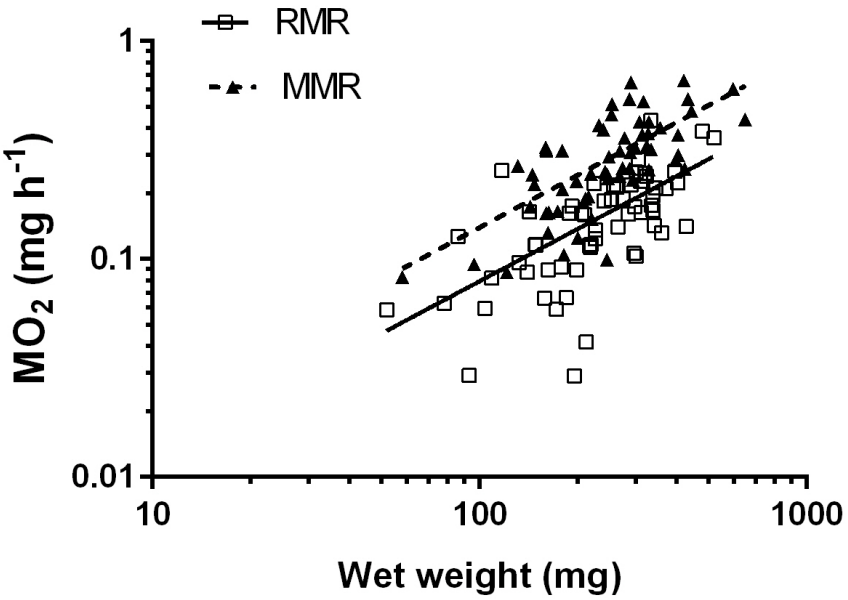

Fig. 1. Fig. R1

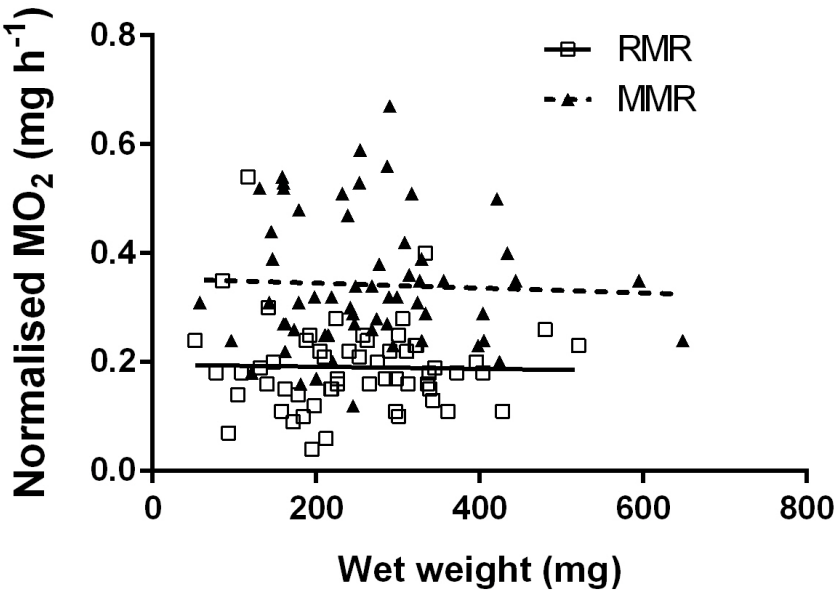

Fig. 2. Fig. R2