Biogeosciences Discuss., 8, C1380–C1385, 2011
www.biogeosciences-discuss.net/8/C1380/2011/

Biogeosciences Discussions

Interactive comment on "Effect of ocean acidification on otolith development in larvae of a tropical marine fish" *by* P. L. Munday et al.

P. L. Munday et al.

philip.munday@jcu.edu.au

Received and published: 2 June 2011

We thank the two reviewers for their very helpful comments and suggestions. We have attended to all these minor comments in the revised manuscript and believe they have significantly improved the final version. Following is a point-by-point response to the comments and how they have been addressed.

Referee # 1 Specific comments: Section 2.1 -Clarification: It was not clear how many larvae were available for these analyses. I note that some of the sample sizes for some parts of the experiment were low (i.e. otolith chemistry analysis).

Response: To clarify we have added extra details of the rearing and sampling process at the end of this section. "Larvae from one breeding pair in the control group, and a

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper

different breeding pair in the pH 7.8 group, did not survive, therefore each treatment contained larvae from at least three of the four parental genotypes. A sample of 10-15 larvae from each clutch was stored in 75% ethanol for otolith analysis. The remaining larvae were stored in formaldehyde for other analyses." Then in Section 2.3 we say "Morphometric measurements were calculated for 16 randomly selected individuals from each treatment and Fast Fourier coefficients were calculated for 19 randomly selected individuals from each treatment (Table 2). Individuals selected in each treatment were representative of at least three different parental genotypes. Samples at each treatment level contained 3-10 individuals from at least 3 different breeding pairs."

Section 2.4 -Again, the sample size used here was not obvious, although it says "one otolith chosen at random from each fish". Figure 3 indicates that the number of samples analyses was quite low? Is there a reason more otoliths were not analysed for their chemistry? An explanation in the text would be useful.

Response: Thanks for picking this up, we have added the sample sizes to this section. Although the sample sizes for chemistry were relatively small (5-7 individuals per treatment), the low variance and consistency of results suggests that a larger sample sizes would not have changed our interpretation in any meaningful way. We have included this explanation in the text.

Discussion -It would have been useful if some of the differences between right and left otoliths that were seen (Table 3) were discussed here.

Response: Good idea. We have specifically added the following text. "A number of studies have shown that physiological stress can increase otolith asymmetry (Gagliano and McCormick 2004; Payan et al. 2004); however, we found no evidence that ocean acidification is likely to decrease symmetry between left and right otoliths, even at relatively high CO2 concentrations. In fact, there was a trend for smaller differences in otolith area and length between left and right otoliths in the highest CO2 treatment, and for there to be less variation among individuals (i.e. lower sample variance). Increased

BGD

8, C1380–C1385, 2011

calcification in the higher CO2 treatment might have tended to ameliorate differences in otolith shape and size, and thus been responsible for the apparent increase in symmetry between otoliths, both within and among individuals. Variation in the size and shape of left and right otoliths may affect the ability of individuals to detect and localize sound (Popper and Lu 2000; Gagliano et al. 2008) and decreased otolith symmetry has been associated with higher mortality rates in larval reef fishes (Gagliano et al. 2008). Consequently, any trend toward increased symmetry, such as that observed here, is unlikely to have a negative impact on individual fitness."

Technical corrections: Section 2.1 -Misspelling: "On discovering a new clutch of egg" Section 2.2 -Millero et al. 2006-not cited in reference section Section 2.3 -"When required" this sounds a bit awkward and maybe it could be rephrased. Section 2.4 -Fitzgerald et al. 2004-not cited in reference section References -There seem to be quite a few errors in the reference section that need to be correct. For example, there are papers listed out of order (the C and D sections have become mixed up), papers cited here but not in the text (Palmer and Srobeck 1986 and Payan et al. 1998) and formatting errors (some papers citing journal articles have the title all in capital letters while most do not).

Response: All technical corrections have been made and the references checked for style and order.

Referee #2 Table 1: some more information on chemistry is needed. For example, What is the level of variability in the measured parameters.

Response: Standard deviations have been added to Table 1 where relevant.

p2335, l11: which anaesthetic was used?

Response: Clove oil anaesthetic was used. This has been added this information, along with an appropriate citation to the method, at the end of section 2.1 "Settlement stage larvae (11-d post hatching) were sacrificed with a lethal dose of clove oil anaes-

BGD

8, C1380–C1385, 2011

thetic (Munday and Wilson 2007)."

M&M: - Is there any information on survival? - p2336, l8-11: I have some trouble to understand how the 16 randomly individuals relates to breeding pairs. Some more details would be useful.

Response: This is addressed in Point 1 from Referee 1, with additional details added to the text.

P2337: relationships between SL and otolith other parameters is very interesting in itself. I would recommend adding these to the manuscript. Are they linear? The size effect is corrected by size but presentation on the results in relation to size would be more interesting (& show regressions).

Response: We are pleased the reviewer raised this point, because in checking the results we found that we had incorrectly recorded a significant relationship for SL and otolith rectangularity for right otoliths. In fact this relationship was high non-significant. We have corrected this error in Table 1. As a consequence, circularity was the only otolith trait that was significant associated with SL. We have added details to the results section that reports this association and added a paragraph to the discussion that deals specifically with this pattern (below). We have not included another figure showing the regressions because SL is simply a covariate in the analysis and there was no effect of treatment on the trait in question (circularity). We do, however, provide the full details of the regression equations in the results section for readers that might be interested. Results: "There was a highly significant effect of SL on otolith circularity for both left and right otoliths (Table 3). Regression analysis revealed a positive linear relationship between otolith circularity and fish standard length (left otoliths: y = 1.457x + 6.507; r2 = 0.517; right otoliths: y = 1.060x + 8.880; r2 = 0.522). Circularity is defined as otolith perimeter2/otolith area. The minimum value of 4pi (12.75) is achieved only for a circular boundary, with values of $\sim$16 for a square boundary and $\sim$ 20 for a triangular boundary. Circularity increased from $\sim$ 15 in the smallest fish (5-6 mm SL) to >20 in the largest fish

BGD

8, C1380–C1385, 2011

(9-11 mm SL) indicating a shift from a square-shaped boundary in smaller fish towards a more triangular shape with increasing fish length." Discussion: "Otolith circularity was the only trait that varied significantly with fish length. There was a linear relationship between SL and circularity, indicating a shift from a square-shaped boundary in smaller fish towards a more triangular shape in larger fish. Otolith deposition does not occur evenly around the otolith, instead, growth often occurs more rapidly on one axis than the other, leading to an otolith that is increasingly more elongate and triangular in shape (Gagliano and McCormick 2004; Green et al. 2009). Otolith growth is often correlated with somatic growth and therefore larger, faster growing fish within treatments may be expected to have a more triangular otolith profile, as observed."

RESULTS: - Data on impact of pCO2 on fish growth would also be interesting.

Response: Results of experiments into the effects of pCO2 of growth of larval clownfishes and other reef fish species has been reported in other papers (Munday et al. 2009 Proc Roy Soc Lond, Munday et al. 2011 MEPS) . Importantly for this study, there was no difference is size among the fish selected for otolith analysis, and we confirmed that within treatment variation in body size did not affect otolith traits before analysis. One trait, circularity, was associated with fish length and was this analysed with ANCOVA. These details are now clearly presented in the results and discussion.

Some pictures of otolith in different pH would be helpful for the reader. - p2341, l7: replace Fig3 by Fig4 - p2341, l15: replace Fig4 by Fig3

Response: Thanks for picking up the error in Figure order, this has been corrected. Our otolith photos are silhouettes, which provide little detail other than general shape. We feel this would add little useful additional information and have chosen not to include a photograph.

DISCUSSION: - It would be nice to have more information on the link between the measured parameters and animal fitness. For example, it is unclear how otolith size and shape evolve with growth and how this relates to function and fitness. Then how

BGD

8, C1380–C1385, 2011

this may be influenced (or not) by pCO2 and if the observed differences could have effect on fitness. - The discussion on life-history is fascinating.

Response: This is a great idea. We have added the following paragraph in the discussion. “Otoliths have an important role in fish hearing and body orientation. However, if the changes in otolith area and maximum length that we observed in the highest CO2 treatment (pH 7.6, 1721 μatm CO2) would be sufficient to affect these important functions is unknown. Otolith size can vary considerably among individual fish of the same somatic size, and otoliths of some species continue to grow even once somatic growth has ceased (Campana 1990; Thorrold and Hare 2002; Munday et al. 2004). Furthermore, we observed considerable variation in otolith morphometric traits among individuals in the control and less extreme CO2 groups. Therefore, some variation in otolith size is commonplace and it is not clear that the relatively small change in otolith size observed here in the highest CO2 treatment would be sufficient to cause substantive effects on larval fish.” Also new text in asymmetry section “Variation in the size and shape of left and right otoliths may affect the ability of individuals to detect and localize sound (Popper and Lu 2000; Gagliano et al. 2008) and decreased otolith symmetry has been associated with higher mortality rates in larval reef fishes (Gagliano et al. 2008). Consequently, any trend toward increased symmetry, such as that observed here, is unlikely to have a negative impact on individual fitness.”

REFERENCE: - The references are not always in alphabetic order.

Response: Corrected

A copy of the revised ms showing changes is uploaded as supplemental material

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/8/C1380/2011/bgd-8-C1380-2011-supplement.pdf

Interactive comment on Biogeosciences Discuss., 8, 2329, 2011.