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Biogeosciences Discussions

Interactive comment on "Short and long term consequences of larval stage exposure to constantly and ephemerally elevated carbon dioxide for marine bivalve populations" *by* C. J. Gobler and S. C. Talmage

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Author's comments for MS No. bg-2012-531:

Short and long term consequences of larval stage exposure to constantly and ephemerally elevated carbon dioxide for marine bivalve populations

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Anonymous Referee #1 Received and published: 8 January 2013 The manuscript of Gobler and Talmage presents data from various experiments aimingat assessing the

effects of ocean acidification on the larval development and the subsequent juvenile period of 2 bivalve species. This manuscript provides very interesting data as it shows direct evidence of deleterious effects of OA on gross calcification rates as measured by the 45Ca incorporation technique, that the early larval stages are the most sensitive to decreasing pH levels, and that larval exposure to low pH has significant impacts on survival and subsequent juvenile growth. I can only recommend this manuscript for publication in Biogeosciences although I would like the authors to consider the following suggestions.

Thank you. We are grateful for the efforts of the reviewer and thank her/him for her/his suggestions. We address the individual reviewer concerns below.

Introduction: P15903, L11: Kurihara studies should not be cited in that context as pH values that were considered were well below the ones expected for the next 100-300 years.

We agree. In a revision, that reference would be removed.

Methods: 2.3. For clarity, please mention for how long the larvae were cultivated at the start of the paragraph.

In a revision, this detail would be moved to the start of the paragraph.

Furthermore, it would have been interesting to provide data on hatching rates and survival for this experiment (until veliger and pediveliger stages).

This data is not available.

How many replicated incubation did you have for 45Ca incorporation? This is unclear as it was mentioned in P15904L16 "(n=4 except for calcium uptake experiments)

There were four replicates. In a revision, this detail would be moved to the start of the paragraph.

Results: 3.2. P15912L7: please change shell length-based by shell diameter-based

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In a revision, this change would be made.

Discussion: P15917L16-17: although this part of the study is very interesting and provides very important data, the experimental setup does not allow assessing the effects of CO2 variations at the same frequency than in estuarine habitats (as mentioned by the authors: tidal and daily fluctuations). The protocol considered exposures to certain levels of CO2 for several days.

We agree. In a revision, we would acknowledge this and emphasize the experimental design does not inform daily tidal fluctuations but rather may match seasonal or sudden. transitions

L25: reference should be Gillikin et al., 2007. In a revision, this change would be made.

Overall, congratulations to the authors.

Thank you.

Anonymous Referee #2 Received and published: 17 January 2013 General comment: This manuscript looks at the effect of both static and fluctuating ocean acidification on the larval and early juvenile development of the bivalves Mercenaria mercenaria and Argopecten irradians. This is an impressive data set which supports many hypotheses suggested but not yet comprehensively supported in the literature. Using calcification, RNA:DNA ratios and growth measurements the authors find that the early larval stages of the two bivalve species are more sensitive to ocean acidification than the later larval and early juvenile stages. The fluctuating pH design of the experiment moves a step closer to mimicking the fluctuating nature of pH in estuarine and coastal environments and shows that exposure to ocean acidification at one life-history stage can have lasting negative effects on others. I would like to recommend the manuscript for publication in Biogeosciences and suggest only the minor changes below.

Thank you. We address the individual reviewer concerns below.

Specific comments: Methods: Excellent and robust delivery and measurement of CO2.

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Section 2.6 – What was the purpose of transferring the juveniles out of the laboratory to the ranging pH environment for 12 days before transferring them to the stable environment? Perhaps include this.

In a revision, we would add the detail that this is a standard hatchery rearing practice.

Results: Section 3.1 Line 22-23 – A. irradians veliger calcium uptake seems to decrease from 280 to 390 uatm but 390 to 750 uatm are the same. This should be made clearer. Also, the difference between veliger and pediveliger larvae could be made clearer i.e pediveliger larvae seem more sensitive to the elevated CO2 level than the earlier veliger stage for this species (in terms of calcium uptake).

In a revision, these details would be explicitly stated in the results.

Section 3.2 – For M. mercenaria, the results of the RNA:DNA ratios are reported for 280 and 1500 uatm, what happened at the other concentrations?

In a revision, these details would be explicitly stated in the results.

Section 3.3 Line 9 – should this be 3, 6, 9 and 12 days? In the methods it says that they were switched until day 12.

The results are correct. Since the methods should read they were switch every three days beginning on day 4 until day 13. This change will be made in the revision.

Discussion: There is not much written about 280 uatm. I know that this is important, showing that the current ambient CO2 levels are already affecting bivalve species but this is not clear from the discussion of results.

Because one of our prior publications extensively emphasized this aspect (Talmage and Gobler 2010), we choose to focus on the findings novel to this manuscript within the discussion.

Technical comments: Introduction: Page 2 Line 4 – Change ‘ecosystem’ to ‘ecosystems’

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In a revision, this change will be made.

Line 17 – The meaning of the second part of this second is not completely clear/ does not quite flow with the start of the sentence. Perhaps modify.

Splitting this sentence into two will fix this problem.

Methods: Section 2.4 – How many days were larvae reared before doing RNA:DNA ratios? I think this may be mentioned below but would be good to include it in this section. Section 2.5

Line 13 – Change 'These' to 'The' Line 19 – Insert 'to' after 'larvae'

In a revision, this change will be made.

Line 22 – Was there a reason for using a different elevated CO2 level for M. mercenaria and A. irradians?

While we measure the carbonate chemistry of our experiments as they proceed, fluctuations in the CO2 levels in ambient air can alter the final CO2 levels achieved within experimental vessels.

Results: Section 3.1 Line 9 – perhaps insert 'on' after 'mercenaria'.

In a revision, this change will be made.

Line 10 – 'M. mercenaria day 10' doesn't quite flow. Perhaps move comma from after '10' to after 'larvae'

In a revision, this change will be made.

Discussion: Page 2 Line 1-2 – Change 'growth of larval growth' to 'growth of larvae'.

In a revision, this change will be made.

Line 7 – Should the full species names be used in this line since it is the first time that the species have been mentioned in this section?

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In a revision, this change will be made.

Line 11-12 – perhaps include a few more recent papers on the effects of OA on bivalve larval development from 2011 and 2012.

Line18 – Add the species in question. i.e. individuals of A. irradians

In a revision, this change will be made.

Line 30 –perhaps change 'exposure to 13 days 750 uatm CO2' to 'exposure to 750 uatm CO2 for 13 days'.

In a revision, this change will be made.

I would like to congratulate the authors on this very interesting and important manuscript.

We are grateful for the efforts of the reviewer and thank her/him for her/his suggestions.

Anonymous Referee #3 Received and published: 17 January 2013 General comments The manuscript by Gobler and Talmage explores the effects of different regimes of exposure to elevated carbon dioxide on the larval stages of the bivalves Mercenaria mercenaria and Argopecten irradians, as well as the carry-over effects to the juvenile stages. The study provides new and relevant experimental evidence on larval survival, calcification rates, RNA:DNA ratios and growth rates. The hypothesis of trans-lifestage 'legacy effects' or "carry-over effects" of exposure to elevated CO2, although not novel, seems to be supported by the dataset presented. The questions addressedare all relevant and well within the scope of the journal. The study is well structured, clearly presented and advances significant contributions to the field, so I recommend its publication in Biogeosciences. There are however, some minor aspects needing revision or clarification, which are detailed bellow.

Thank you. We are grateful for the efforts of the reviewer and thank her/him for her/his suggestions. We address the individual reviewer concerns below.

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Specific comments Methods: p.15906, L.12: The origin of the larvae used in the experiments is not given. Describe briefly how they were obtained.

All larvae were obtained from broodstock collected from mesotrophic estuaries (eastern Peconic Estuary) that experience a modest range in pH and pCO2 (pH = 7.9-8.2; pCO2 = 300 – 500 μatm, C.J. Gobler, unpubl.) and spawned at the East Hampton Town Shellfish Hatchery. In a revision, this detail would be added.

p.15906, L.15: What was the density of T-iso cells provided?

4 x 104 cells ml-1 daily. In a revision, this detail would be added.

p.15906, L.22: It is not clear what the expression "1% of its original concentration" refers to. Please clarify

The stock solution was diluted 100-fold. In a revision, this detail would be added.

p.15906, L.25-28: Given that no difference in larval performance was observed in experiments done with and without antibiotics I do not understand the rationale for using antibiotics.

This was discovered after this experiment had been completed and thus too late to affect these experiments.

p. 15908, L.7-8: The assumption about the relative importance of shell dissolution and deposition seems questionable. In fact, in previous studies by these authors (Talmage and Gobler, 2009, 2010), larval bivalve shells exposed to elevated concentrations of CO2 have been considered highly vulnerable to dissolution. Please clarify this issue in the discussion.

In a revision we would acknowledge this and state that these should be considered net calcification rates.

p. 15909, L.25-27: Were 40 individuals used per treatment or in total? According to Figure 5, four replicate beakers were used before and after the transfer across treat-

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ments. Please clarify.

In a revision we would clarify that is was 40 individuals used per treatment.

p. 15910: How many experimental units and how many individuals per unit were used for each treatment level in the long term growth experiment.

In a revision we would clarify that is was one experimental unit for all treatments and 200 individuals used per treatment.

p. 15910, L23-24: The purpose of the ranking procedure prior to data analysis is not clear. Were non-parametric statistics used?

In a revision, we would indicate that an ANOVA on ranks is a non- parametric statistical test.

Results p. 15914, L6-7: The stated objective here is was to measure post- settlement growth, but results are given for two post-spawning periods. This is confusing because, if I understood correctly, the first measurement for the 12 week period was done while the individuals were exposed to the different CO2 treatments and the second measurement was done after the transplants to the field. In contrast, both measurements during weeks 13-26 were already done in the field.

There was only one spawn. In a revision, we would indicate that the first measurement for the 12 week period was done while the individuals were exposed to the different CO2 treatments and the second measurement was done after the transplants to the field. In contrast, both measurements during weeks 13-26 were already done in the field.

p. 15914, L14-15: According to Figure 7, in September the shell diameter at 390 uatm was approximately 16 mm. Individuals reared at 390 and 750 _atm were probably already similar in size by January.

In a revision, state these details in the results.

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Discussion p. 15915, L2-4: I think some clarification on the experimental design of the long term growth experiment is needed to support the claim about the carry-over effects (see comments above on the methods section).

In a revision, we would clarify the experimental design as outlined above.

p. 15916, L7-9: Given the methodological assumption that shell dissolution is negligible relative to shell dissolution, the results observed here should be largely attributed to reduced accretion of new shell. Please clarify.

In a revision we would acknowledge this and state that these should be considered net calcification rates.

p. 15919, L3-4: The conclusion about the faster growth of juvenile individuals exposed to high CO2 as larvae is confounded by the fact that, although the individuals were transplanted to the field after 47 d, growth was measured from 0-12 weeks. See comment above (p. 15914, L6-7).

We do not believe this confounds our results.

p. 15919, L19: On carry-over effects, see also: Parker LM, Ross PM, O'Connor WA, Borysko L, Raftos DA, Pörtner H (2012) Adult exposure influences offspring response to ocean acidification in oysters. Global Change Biology 18:82–92

Dupont S, Dorey N, Stumpp M, Melzner F, Thorndyke M (2012) Long-term and trans-life-cycle effects of exposure to ocean acidification in the green sea urchin Strongylocentrotus droebachiensis. Marine Biology: 1–9. DOI 10.1007/s00227-012-1921-x

We thank the reviewer for pointing out these papers. In a revision, we would cite these within the discussion.

Interactive comment on Biogeosciences Discuss., 9, 15901, 2012.