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Interactive comment on “Fertilization success of an arctic sea urchin species, *Strongylocentrotus droebachiensis* (O. F. Müller, 1776) under CO₂-induced ocean acidification” by D. Bögner et al.

Anonymous Referee #4

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Manuscript Revision: “Fertilization success of an arctic sea urchin species, *Strongylocentrotus droebachiensis* under CO₂-induced ocean acidification” by D. Bögner, U. Bickmeyer and A.Köhler for Biogeosciences Discussions (10, 8027-8064, 2013)

General comments

The present study investigates the effects of CO₂ induced ocean acidification on fertilization and early development in the sea urchin *Strongylocentrotus droebachiensis*. In general the study is well designed and conducted appropriately. The approach of studying the effects of pre-incubation under elevated CO₂ conditions on the response

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of fertilization in sea urchins is interesting and novel. Although the data can be a nice contribution towards the understanding of OA impacts on fertilization in sea urchins, the way they are presented makes it difficult to access the results and its main conclusions and I regret that I have to recommend the article, as it is now, to be rejected for publication in Biogeosciences. The pure language as well as missing information regarding the methods makes it difficult to assess the quality of the scientific approach and its outcome. Most of the chapters are much too long. The manuscript should be thoroughly revised with respect to language and should be shortened wherever possible. There are major flaws in the analysis as well as in the conclusions the authors draw from the results. Please find my more specific comments below. I hope that some of them will be helpful in rewriting the manuscript for future publication.

More specific: It is not clear from the manuscript why the authors use 380 μatm and an additional control in their experimental settings. The methods, in general, are missing information to better evaluate the quality of the experiments. There is no sperm concentration given that was used in the experiments. The authors mention throughout the manuscript that the 3000 μatm treatment had a higher aeration, a higher salinity as well as a higher temperature compared to all other treatments. This needs to be clarified and thoroughly discussed in the manuscript. The inclusion of salinity as a factor in the statistical model does not solve the problem (please find my specific comments below). The manuscript is far too long and many of the issues debated in the discussion chapter are not related and supported by the data obtained from the study; appropriate references are missing throughout the manuscript.

Specific comments

Abstract

8027/6-7 It is not clear why the authors aerated the filtered seawater with fixed partial pressures of 380 μatm and additionally use “untreated filtered seawater” as control.

The authors talk about fertilization rates but “rates” were not measured or are at least

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not presented.

8027/18 The authors do not need to give the abbreviation in the abstract since this term is not used in the abstract again.

Introduction

8028/2 Give references for OA impacts on “calcifying animal groups”.

8028/8 Must be “broadcast spawning”.

8028/9-10 Better “, such as fertilization and post-larval development”.

8028/13-21 It might be better to pick out some references most relevant to the investigated group of organisms or to the methods applied in the present study. If the authors want to give an overview of the available literature on OA, better cite the most recent reviews and meta-analyzes, e.g.: Kroeker et al. 2010, 2013; Harvey et al. 2013; Dupont et al. 2010.

8028/24 Why are they of high value, please clarify.

8028/27-28 “Until now published, results on fertilization experiment . . .”, please rewrite.

8028/30 Give references.

8029/4 “no data exist”? Better “no data on fertilization success of *Strongylocentrotus droebachiensis* under elevated pCO₂ exist from the Arctic”. Be careful with these statements.

8029/6-7 What does this mean for this ecosystem? Why is it assumed to be more vulnerable to future OA? Are there other important issues making this region more vulnerable? Please clarify.

8029/10-12 Here the authors state that this species is widely distributed but in most parts of the text the authors are speaking of an “arctic species”. Please rephrase.

8029/15-21 Are there any data available that describe pH fluctuation in this area? This

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would put the study in a better ecological context.

8030/1 Better “takes place”. This is just one of many typing errors, which makes the text difficult to read.

8030/10 The abbreviation should be given in the first mentioning of the main text body. Thereafter the abbreviation should be used only.

8030/17 Again, what is the difference between 380 μatm and untreated filtered seawater?

8030/18 At the first mentioning of “BCECF/AM”, this term should be described for people unfamiliar with this abbreviation.

8030/14-19 Could be moved to the Materials and Methods.

Materials and Methods

8031/5 Seeing the distance between Kongsfjordneset and the AWI I consider “short” a vague term. A better description of the transport conditions is necessary.

8031/7 What was the natural light? Could be easily mentioned here.

8031/8 I guess “EHEIM ecco pro filters” are biological filters? Please mention in this case.

8031/14 What was the natural salinity in which the specimens were collected? And what does this mean to the animals? Could they have been stressed?

8031/21 Why is the 3000 μatm treatment conducted at a different temperature? Was this conducted at a different time, also? Please clarify.

8031/26 I do not understand why you used 380 and additionally seawater without CO₂ manipulation. Isn't 380 considered the control in this case? Please clarify the difference between both levels and the relevance of using an extra “control”.

8032/5 Better “We measured”.

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8032/12 Why only of “control seawater”?

8032/12 From what exactly were the values calculated? From AT and DIC or AT and pH or DIC and pH?

8032/23-24 Were the observations done manually under a microscope? If yes, please state so.

8032/27 Please clarify ASW and ASW when first mentioning.

8033/1 This is no concentration. Please clarify.

8033/12-17 The use of 25 ml and 100 ml vials makes it difficult to directly compare the WOPI and WIPI experiments. Please state that at least all other conditions (sperm concentration . . .) were constant between the two experiments.

8033/21 Use “experiments” rather than “tests”.

8034/17 Are there any references for this method?

8035/18-21 Were the data tested for normality or for homogeneity of variance? If yes, state so and give the method used.

Results

8035/24-25 Delete the first two sentences.

8036/15 Why did the authors not use the actual measured values for analyses as well as for the graphs. They could use either pH or pCO₂. This is more relevant since organisms were actually exposed to the measured not to the intended conditions.

8036/17-21 How do the authors conclude this and tell salinity from CO₂ effects apart? There was just one salinity within the 3000 μ atm treatment. So including salinity in the model would not solve this issue. I recommend the authors to include a statement in the discussion that a possible salinity effect or effects from higher flow-through cannot be separated from the CO₂ effect but that a salinity effect is unlikely due to the small

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differences in salinity between the treatments. Another issue that should be mentioned at some point are the differences in temperature for the 3000 μatm treatment compared to all others (see 8031/21).

The entire results part is still confusing and needs careful revision and shortening. One example would just be the following (8036/22): instead of writing “Figure 4 shows the distribution of the described morphotypes in relation to treatment levels. Analyzing data from all experiments without discerning on experimental approach, we observed that pH changes had clear effects on the proportion of eggs grouped as perfect FE formation (PFE) (which decreases at higher pCO₂ levels) and on the proportion of polyspermic eggs with no FE formation (NFE) (that increases at higher pCO₂ levels).” the authors should for example integrate the reference to the figure in the following way: “The factor pH had clear effects on the proportion of eggs grouped as perfect FE formation (PFE), decreasing with higher pCO₂ levels and on the proportion of polyspermic eggs with no FE formation (NFE), increasing with higher pCO₂ levels (Fig. 4)”. This is just one example on how the results could be reduced in length and sharpened to the point.

There might be different more appropriate ways to evaluate the OA impacts on early development than just mentioning the observations in the text (the proportion of zygotes that reached a certain stage at a defined time point, for example).

Discussion

8038/24 Why do the authors state this here: “at the cellular level”?

8039/17 What is the meaning of abbreviations if words such as “hyline blebbing (HB)” are abbreviated and explained the same time at so many occasions throughout the manuscript? Only abbreviate the most commonly used expressions and use full spelling of the others.

8040/1-2 Give a reference.

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8042/21-8043/10 The entire paragraph focuses on an issue that was not even addressed in the present study and should thus be reduced.

8044/6 This is the first time the authors mention this possibly very interesting finding. It should, if used in the discussion, mentioned in the results before and explained in more detail.

8046/7-10 Leave out.

8046/14 Not supported by the data. Leave out or give reference.

At many occasions the discussion goes much too far into detail and into hypothetical thinking for the data obtained in the experiments and their relevance. Please revise the discussion with respect to this.

Figures and Tables

Table 1 It is not clear from the table whether the carbonate system changed over the cause of the experiments. Furthermore it would be useful to give values of calculated pCO₂ as well as saturation states. The authors do not need to give the “n” in the table but rather mention it in the table legend, e.g.: “n=33 for the 3000 μatm treatments, n=44 for all others”.

Table 2 This table should be referred to in the Materials and Methods section, not in the Results.

Table 3 Redundant! Abbreviations need to be only clarified at the first mentioning in the main text body and if applicable in table or figure legends. In any case, try to avoid too many abbreviations.

Fig. 1 and Fig. 2 Could be supplementary.

Fig. 3 line 2 Rather than including SF and UF in these graphs it might be worth considering to remove one of the two, since both are totally dependent on each other. This would open up space for including the WOPI and the WIPI results in one graph.

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This might strengthen the focus on the comparison of both approaches, since this pre-incubation is a novel approach in this study. The way of illustrating significant differences from pairwise comparisons of the different treatments seems not intuitive. There are other ways to illustrate this: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; to just give one example.

Fig. 4 Why do you give “100x magnification”? It is totally irrelevant to this graph. SF and UF (the first two graphs to the left) only show a reverse picture of the results. It might be worth to consider removing one graph, since both are totally dependent on each other.

Supplementary Material

Please give a better figure legend to these figures.

More general comments

It never became really clear how many real independent replicates the authors used in their experiments?

The authors use pH and pCO₂ at many different occasions inconsistently throughout the manuscript. Deciding on one variable and using this consistently in all chapters and graphs would make it easier for the reader to follow.

Interactive comment on Biogeosciences Discuss., 10, 8027, 2013.

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