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Interactive comment on "Fertilization success of an arctic sea urchin species, *Strongylocentrotus droebachiensis* (O. F. Müller, 1776) under CO_2-induced ocean acidification" *by* D. Bögner et al.

Anonymous Referee #6

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The present study evaluated the effects of OA on the fertilization process of the sea urchin *S. droebachiensis* by pre-incubating eggs in different CO2 conditions. As a result, it was found that the fertilization rate decline with the increase of pre-incubation time and CO2 concentration.

Although present study includes some new findings, this paper is not acceptable in the present status because the methodology and the results of the present paper seems to be not fully sounds and hence it was impossible to validate the results and conclusion. For example, though present study aim to evaluate the effect of pre-incubation time

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Interactive Discussion
Discussion Paper

in OA condition on the sea urchin early development, the experimental methods and results are not clearly demonstrated. The statistical analysis is also not clear and the several discussions seem to be not justified by the present result.

Following are the specific comments:

p. 8029 line 14-21 Since the present study is focused on “early development of sea urchin”, this long list of reference would be more informative if authors focus on studies evaluating the effects of OA on early development of marine organisms (or echinoderm). Information about the already known effect of OA on sea urchin early development would help to clear the aim of the present study.

p. 8029 line 24 All references should be re-order from previous to recent studies throughout the paper.

p. 8029 line 25 What you mean by “cellular level”?

p. 8029 line 29 What kind of variation in experimental methodology exist between studies that can be “critical”?

p. 8030 line 1 Again, what kind of difference in fertilization protocol which limits the comparison between studies exist, and how the present study overcome these limitations?

p. 8030 line 18 “photosynthesis” is not the unique process that affects pH

p. 8031 line 9 Background about the change of intracellular pH during fertilization would be informative.

Material and Methods

p. 8032 line 3 Please describe the latitude, longitude, depth, date and number of sea urchins that have been collected.

p. 8032 line 14 Please describe in more detail from where the seawater was collected

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and the seawater chemistry of the seawater.

p. 8031 p. 1 What was the flow rate for 3300 atm? Why the flow rate for this concentration was different from the others?

p. 8033 p. 5 What you mean for controlling alkalinity? Have you add any base or acids?

p. 8033 line 8 When the seawater TA, DIC and salinity was measured? Just after bubbling the seawater or just before the experiment was conducted?

p. 8033 line 14 How long the bubbled seawater was kept in the bottles before using to the experiment?

p. 8033 line 19 What you mean for each fertilization? You mean that the fertilization experiment was conducted many times? If yes, how many times the experiment was conducted? How many sea urchins were used for each experiment? Are the egg and sperms of 4-6 females and 2-4 males were mixed? If yes, what you have treat as "replicate"?

p. 8034 line 5 What this fertilization proceed for 1h and 3h mean? Do you mean that the fertilized eggs were fixed 1h and 3h after fertilization? If yes, what was the purpose of having these two different fertilization time. Additionally, I could not find the results of this experiment. I also could not find any discussion in regard with this point.

p. 8034 line 13-18 The experimental method of this experiment is not clear. The WOPI (without pre-incubation) and WIPI (PI.t = 0h) are different? Is that mean that one experiment without any incubation using 25ml bottles were conducted and a separated experiment with three different incubation time (0, 1 and 3h) was conducted using 100ml bottles? If yes, why authors needed to conduct the WOPI experiment in addition to the WIPI (PI.t = 0)? These experiments were conducted in set using the same sea urchins (4-6 females and 2-4 males)? How many bottles were used for each CO2 condition in WIPI experiment? How many eggs (or what was the egg concentration)

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were included in each bottle? Which figures show the result of which experiment? For example, Fig. 1, 2, 4, 5 are pictures and data of which experiment? Fig 3 show data of I: all experiment, II: WOPI exp and III: WIPI exp. What you mean for all experiment? If this is the data of WOPI and WIPI, how authors could combine these results even though the methodology is different. Are the data of WIPI 0, 1 and 3h are all combined at Fig. 3III? If yes why? For my understanding the authors aimed to evaluate the effect of pre-incubation time on the fertilization rate of the sea urchin. Where can I found these results?

p. 8034 line 18 Is that mean that there are 3 bottles x 2 Ft time (1 and 3h) X 6 CO2 condition $=$ 36 for WOPI and 3 bottles x 2 Ft time (1 and 3h) X 3 pre-incubation time X 6 CO2 condition $=$ 108 for WIPI?

p. 8035 line 10 The experimental methodology of larval experiment is very unclear. Is this experiment was conducted independently of WOPI or WIPI experiment? I expect that the sea water chemistry (pH pCO2) will be highly modified by the respiration of larvae and respiration $+$ photosynthesis of phytplankton without any change of sea water during 72h. Have you measure the seawater pH or TA and DIC after the incubation? What mean 50μL?

p. 8035 17 Though BCECF-AM can be a very strong tool for measuring pHi, lot of care should be taken for using this methods. First of all you should show that in your experiment there is really a correlation between pH and 490/439 Ratio. Even if you have not shown the exact pH, you first have to give a justification for the accuracy of this methodology.

p. 8036 line Statistics analysis is very unclear.

Results:

All figures are very hard to interpret since it is unclear which results are shown in which figure. For my understanding at least there are experiment with and without incubation,

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for the experiment with incubation have 3 different times, and there are also 2 different fertilization time. Where we can see all these results? What is the number of error bars in each figures. Please explain the definition of your replicates. Please add ANOVA table for all the results. As mentioned before, I could not understand from which figure I can see the effect of pre-incubation time on the fertilization rate, which is thought to the most important figure.

Authors make a big effort to try to categorize a lot of type of "polysperm" by the image of the eggs, authors should also show any biological justification for these categorization.

Discussion

The discussion is mostly focused on the effect of OA on polysperm and fertilization process, however it is hard to accept without justification for the correlation between each fertilization process and the classified image of the eggs.

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