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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.

L. Santella (Referee)

santella@szn.it

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Comments on: Fertilization success of an artic sea urchin species, Strongilocentrotus droebachiensis (O. F. Müller, 1776) under CO2-induced ocean acidification, Bögner et al. (2013). Biogeosciences Discuss. 10, 8027-8064.

General Comments:

This paper discusses the effect of the ocean acidification (higher sea water pCO2) on the fertilization success of the arctic sea urchin species Strongilocentrotus droe-

bachiensis. The topic is important, as many marine invertebrates spawn their gametes for external fertilization, and have thus been used to study the effect of increased acidification of the medium on the development of the embryo, which has been shown to be sensitive to it. As stated by the Authors, this is the first paper addressing the effects of CO2-induced ocean acidification on the early events of fertilization (sperm egg interaction and cortical granules exocytosis). According to the prevailing view, a successful monospermic fertilization in sea urchin eggs is normally assessed by the elevation of the fertilization envelope (FE) as a result of the exocytosis of the content of the cortical granules into the perivitelline space, which mechanically blocks the entrance of supernumerary sperm. The Authors have classified the fertilized eggs with respect to the morphology of the cytoplasm, the FE, and the hyaline layer.

Interestingly, the Authors have found that the exposure of unfertilized eggs to acidification levels of 1400 μatm and 3000 μatm and to CO2-induced ocean acidification significantly impaired the morphology of the activated eggs. A description of egg categories, classified as successfully and unsuccessfully fertilized, is given in Table 2. It shows that the unsuccessful fertilization is represented by eggs/zygotes that underwent dramatic alterations of their structural organization. In this context, it is appropriate to mention that Ernest Everett Just (The Biology of the Cell Surface, 1939) had shown that eggs of Arbacia, which were never polyspermic, became polyspermic when the ectoplasm (the cortical region of the egg) was injured. This suggestion is in line with recent studies on another echinoderm (starfish) indicating that the alteration of the structural organization of the subplasmalemmal F-actin renders the egg surface receptive to supernumerary sperm (Puppo et al PLoS ONE, 2008).

Furthermore, it has been shown that a proper rearrangement of the actin cytoskeleton during fertilization of starfish eggs is essential for the cortical granules exocytosis and the normal elevation of the fertilization envelope (Kyozuka et al. Dev. Biol. 2008; Chun et al. PLoS ONE, 2010) and that the alteration of the cortical actin cytoskeleton by ionomycin may induce a monospermic fertilization in the absence of the elevation of the fertilization envelope (Vasilev et al. PLoS ONE, 2012). The Authors have indeed discussed a possible role of intracellular pH in the control of the assembly/disassembly of the actin filaments at fertilization.

Specific points:

Fig. 2 The Authors have grouped the eggs into 3 categories: In (b) they represent a polyspermic zygote in which the fertilization envelope has not been elevated. This is an overstatement as it derives from the morphological observation of an abnormal embryo cleavage. However, there is no evidence that this is due to the entry of supernumerary sperm. The alteration of the structural organization of cortical actin has been shown to prevent the entry of the sperm in eggs that had been activated by the latter (Puppo et al PLoS ONE, 2008). A direct link between the lack of FE elevation and a polyspermic fertilization is missing. The authors should label the sperm to show the presence of more than one sperm inside the egg. As mentioned above the inhibition of the FE elevation does not guarantee a monospermic activation of the egg (Vasilev et PLoS ONE, 2012).

(d-i) illustrate the morphological aberrations of polyspermic zygotes in which the FE has been formed. Again, this is an important point: panels h and i show a large number of sperm in the perivitelline space. How could supernumerary sperm have traversed the fertilization envelope? What about the role of the FE in mechanically blocking the entry of supernumerary sperm? And are the Authors sure that many sperm also entered the egg?

In summary, this contribution indicates that the exposure to acidic conditions may have significant effects on the structural organization of the eggs of this sea urchin species. The data of the contribution show significant effects of the increased acidification of the medium on the cell biology and physiology of the eggs at earlier stages of the fertilization process. Further studies on the modifications of the structural organization of the actin cytoskeleton of the eggs induced by an increased acidification would be

necessary to overcome the deleterious impact on development of ocean acidification, which represents a major threat to calcifying larvae of many marine invertebrates.

Luigia Santella

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/10/C2681/2013/bgd-10-C2681-2013-supplement.pdf

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