

## ***Interactive comment on “Ocean acidification reduces mechanical properties of the Portuguese oyster shell with impaired microstructure: a hierarchical analysis” by Yuan Meng et al.***

### **Anonymous Referee #2**

Received and published: 20 June 2018

Review BG-2018-204 Basic reporting In this study, the authors placed oysters from the genus *Crassostrea* in a range of 4 pCO<sub>2</sub> scenarios to establish the quantitative relationship between microstructural and mechanical properties of juvenile oyster shells under increased OA conditions. The authors investigated into structural and mechanical properties using the SAM, the EBSD and nanoindentation tests. It is a straight forward paper, relatively well written and critical in filling gaps of current knowledge on the hierarchical structural organization of oyster shells under elevated pCO<sub>2</sub> conditions. However, I have a few concerns regarding especially the methods and discussion that the authors should consider and address prior to publication.

C1

#### Major comments:

The supplementary table should be placed within the main text, this is valuable information showing robustness of experiment.

A figure illustrating a schematic of the pCO<sub>2</sub> system set up with tanks should be added to methods to improve reader's understanding.

Was growth monitored (and did it differ with pH)? This may be important to deepen the discussion: could it be that differences in crystallography are essentially due to (impaired) growth or does the process of calcification (e.g. calcification rates) appear not be hampered and are most of the differences imprinted after shell formation?

It is a shame that not a few measurements were done on specimens collected from the field. This would have allowed the authors to check whether the shells formed in the experiment are representative (crystallographically) of those found in nature.

#### Minor comments:

##### Title

I think 'reduction of a property' is a bit meaningless. Consider changing it into: '...reduces hardness and stiffness of the...' or something similar.

##### Abstract

line 16: please remove 'coastal areas' or rephrase. Particularly near-coast, OA is hard to detect due to the relatively large fluctuations in inorganic carbon chemistry in such environments due to seasonality, river runoff, sedimentary geochemistry, etc.

line 19: have been very well documented (not has)

line 22: see comment to title

line 22 and further: please mention here that your study deals with juvenile oysters (<35 days old). Previous studies have shown that juveniles may be affected differently

C2

(usually more severe) by OA than adults.

Line 23: shell takes an "s"

Line 31: she's defensive function

Line 31: "surfaces" not used correctly (shows?)

Introduction

Line 35: change belong for belonging

line 38: I don't see how calcite is relatively brittle. It is, for example, more resistant to dissolution.

line 40: please delete 'fascinating'

line 45: protect takes an s

line 52: shells "developing" under... add word

line 53: dissolution occurred in Ries et al. (2011) high CO<sub>2</sub> scenario due to the saturation state being less than 1. Oceans' Arg and Calc saturation states are quite far from being lower than 1 even with ongoing ocean acidification. I would remove this part or mention dissolution only occurred in very high CO<sub>2</sub> scenario.

Line 61: correct the word "demonstrate"

Line 62: Stating "elevated CO<sub>2</sub> conditions" is self-explanatory to how it affect the carbonate system. Remove "and OA"

Line 62: correct "structural"

Line 67 and throughout the text: this phrasing "high CO<sub>2</sub> induced decreased ph" is a not very elegant. Replace by something like high CO<sub>2</sub> scenarios/treatments. The decreased pH is implied.

Line 66 + 69: repetition specifically

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Line 69: materials science techniques?? Correct sentence

Methods

line 75 and on: how many specimens were incubated? How many survived/ grew into maturity? Did pH have any effect on the mortality?

Line 77-78: bad wording. They were left to acclimatize in flow-through...

line 78 and further in the manuscript: salinity is unitless, so please remove 'psu'.

Line 82: remove word process

Line 96: oyster takes an s

line 102: was pH measured daily? Please include the 'n' in the (suppl) table.

line 105: TA was measured every four days, although the supplementary table indicates that TA was calculated.

line 107-110: the calculated inorganic carbon parameters are accompanied by error estimates. How were they calculated?

Line 129: remove word "of" before "greater"

line 128-130: Is the 'thresholding' susceptible to settings (i.e. contrast) of the SEM?

Line 156: not clear. Average per specimen?

Line 157: not clear, why not compare all values?

Results

Line 180: correct Decreased pH in title

line 183: 'erosion' or 'physical damage' sounds as if the formed prismatic layer was intact at first and later dissolved or damaged. Is there any evidence for this or could it also be that the calcification of the prismatic layer was hampered to begin with?

C4

## Discussion

The fact that many oyster larvae were capable of producing new foliated calcite at undersaturation (at pH 7.2) is highly interesting and although the authors are not the first ones to show this, discussing this result may improve the manuscript.

I miss references to some papers dealing with the crystallography of bivalve shells (below), which may help to compare the overall patterns found here with those published previously (i.e. in addition to their between-treatment comparison).

Dauphin and Denis, 2000. *Comp Biochem Phys A*, 126: 367.

Krause & Nehring, J., Klügel, A., Nehrke, G., Brellochs, B., & Brey, T. (2011). Impact of sample pretreatment on the measured element concentrations in the bivalve *Arctica islandica*. *Geochemistry, Geophysics, Geosystems*, 12(7).

Line 309-312: You state that previous studies have shown that on *C. gigas* and blue mussel increase their shells strength and size under higher CO<sub>2</sub> levels, this should be in main discussion and more explanation to why this may occur. You mention that the pCO<sub>2</sub> level is 100  $\mu$ atm which is twice and 4 times lower than your higher treatments.

Line 233: revealed

Line 237: they are many more recent papers

Line 245: remove word "is"

Line 264: bad wording, "reduces with" instead of has started reducing

Line 268: bad wording. "reduces with" instead of has started to reduce

Line 273: replace run by "occur throughout"

Line 309: wrong use of indeed, remove please.

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