

Interactive comment on “Effect of ocean acidification and elevated temperature on growth of calcifying tubeworm shells (*Spirorbis spirorbis*): An *in-situ* benthocosm approach” by Sha Ni et al.

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This manuscript entitled "Effect of ocean acidification and elevated temperature on growth of calcifying tubeworm shells (*Spirorbis spirorbis*): An *in-situ* benthocosm approach" authored by Sha Ni, Isabelle Taubner, Florian Böhm, Vera Winde, and Michael E. Böttcher examines the impacts of ocean acidification and warming on the calcifying tubeworm that grows on the *Fucus* algae in four seasonal experiments.

This study investigated the newly calcified materials of the tube worm using tube di-

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mensions, net growth, microstructures under SEM, and electron microprobe detection of Ca density. The bethocosom setup provided naturally varying carbonate chemistry in seawater to the organisms for the experiment. This work suggests a seasonal trend of calcification related to maximum irradiance and the respective response to ocean acidification.

Unfortunately, these seasonal observations were not replicated and should be interpreted with caution.

Major comments:

This work has a lot of merits, for examples, the experiment was nicely controlled, the interaction of the study species with the algae is very interesting, the manipulated pH and temperature were both environmentally realistic, and the authors demonstrated a good level of understanding on the growth of tube. The tested levels of pCO₂ and temperature treatments can help to better understand the impacts of Ocean Acidification on growth and calcification. However, the current statistical approach is prone to misleading conclusions on the effects of season and treatment. Firstly, the lack of sound independent replication season limits the conclusions that can be made. In addition, the approach in statistical analysis may overestimate the treatment effects from oversampling pseudoreplicates. Therefore, the authors should carefully modify their interpretations and make sure their observation is supported by sound statistics.

This work has only studied one year to understand potential seasonal impacts, however, authors tried to make a conclusion on seasonal impacts (i.e. Spring, Summer, Autumn, Winter) on the observed responses. This is not appropriate unless there were more than 3 years of observation to make each of spring, summer, autumn, and winter to have 3 independent and random observation. The authors are reminded that any seasonal effect observed is a suggestive trend for future experiments.

The interpretation of the result is not appropriate. The experiment is designed to appropriately answer whether there were effects by the 4 levels of treatment (ambient,

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pCO₂, +T, and pCO₂+T), each benthocosm served as independent replicates for statistical analysis. HOWEVER, samples taken within the samples mesocosm are NOT independent replicates. I suggest the authors take the average value for every parameter they measure as the value in each replicate and perform 2-way ANOVA or appropriate statistical test, take n=3. Over-sampling and pseudoreplication may lead to an overemphasis of the effects of treatment effects.

Discussion: This study evaluated the *S. spirobis* tubes in the natural growing scenarios in the presence of *Fucus* substrate. It is important to address a more general theory how calcifiers may benefit from the presence of photosynthetic activities of macroalgae in the other ecosystems.

Furthermore, the impact of pCO₂ on the photosynthetic activity of *Fucus* is also an important point of discussion. Here are a few studies which are relevant to this topic of discussion.

Bordeyne, F, Migne, A, Davoult, D. (2015) Metabolic activity of intertidal *Fucus* spp. communities: evidence for high aerial carbon fluxes displaying seasonal variability, MARINE BIOLOGY: 162 (10): 2119-2129 DOI: 10.1007/s00227-015-2741-6

Raddatz, S, Guy-Haim, T, Rilov, G, Wahl, M (2017) FUTURE WARMING AND ACIDIFICATION EFFECTS ON ANTI-FOULING AND ANTI-HERBIVORY TRAITS OF THE BROWN ALGA *FUCUS VESICULOSUS* (PHAEOPHYCEAE) JOURNAL OF PHYCOLOGY 53 (1): 44-58 DOI: 10.1111/jpy.12473

Detailed comments:

Figure 5A, 12, 13, and 15, should be removed or plotted again after inappropriate interpretation of seasonal response and pseudoreplication are eliminated.

Page 6 Line 2 This selection of subpopulation needs to be further justified

Page 12 Line 5-9 how did the researcher ensure the growth measurement of broken and strongly damaged tubes was accurate?

Page 4 Line 9 "In this area,"

Page 4 Line 23 How to quantify the volume of the blades, what has been done to ensure they are similar volume? How many worms were present in each replicate tank?

Page 4 Line 26-27 provide the size/ volume of the boxes and duration of staining, at what density were the animal kept in the staining solution? Was the pH value before and after the staining monitored? Please provide this information for repeatability.

Page 5 Line 11 "In total,"

Page 6 Line 2 provide statistics for the difference

Page 6 Line 16 list polishing materials and duration for reproducibility

Page 6 Line 21 please provide excitation and emission wavelength, and resolution of images acquired

Page 6 Line 27 pH calibration was at the lower range (pH 4 and pH 6.865), this requires extrapolation when measuring the ocean pH at 7.4-8.0. it is recommended to check the accuracy of this approach with another meter that has been calibrated by 3 points (for example pH 4, 7 10)

Page 6 Line 31, what is the importance of filtering and adding NaCl to TA samples? Please supplement the reason.

Page 7 Line 1 should it be "Both DIC and TA measurements are calibrated. . ."?

Page 7 Line 13 what was the technique used for measuring Ca Si and P?

Page 7 Line 30 it is clearer to say " with respect to *S. spirobis* tube Mg-calcite"

Page 9 Line 6-21 The use of adult and juvenile is not appropriate if the literature shows that sexual maturity begins at 1.9m. Authors are recommended to use neutral words such as small and large cohort/ young and old cohort

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Page 9 Line 22-29 Please explain the use of "mode" instead of "mean"

Page 10 Line 15 Please reword and clarify the meaning of this line

Page 10 Line 21 "Tube opening" not "tube mouth"

Page 10 Line 24 and Figure 10 Please provide sample size that supports this percentage measurements

Page 12 Line 23 to Page 13 Line 6 The seasonal effects is not supported by independent replication.

Figure 1 please add arrows to show juvenile and adults

Figure 2 Please make the font bigger

Figure 7 Please improve the font choice for "Growth direction" and " Tube wall inner surface", it is not easy to read

Figure 8 The labels for the longitudinal section and the cross section are mixed up, note that longitudinal section is the cut made along the long axis of the tube.

Figure 9b - please added annotation to indicate the microboring structures as mentioned in Page 11 Line 12

Figure 12 use of red triangle and red circle makes the pattern hard to see, n=2783 is an example of pseudoreplication, please re-examine the result and make a new plot

Figure 16 fonts for the scale bar are too small

Table 2 - was is the total number of worms sampled for this percentage? Please provide the numbers by expanding the table.

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