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Biogeosciences Discussions

Interactive comment on "Effect of Ocean acidification on growth, calcification and recruitment of calcifying and non-calcifying epibionts of brown algae" *by* V. Saderne and M. Wahl

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We wish to thanks the three referees for their comments that will help greatly to the improvement of the manuscript. Indeed, all three emphasize the needs for the MS to be more focused, more streamlined, more accurate in the hypotheses and the methods descriptions and more concise in the discussion.

Title:

Referee #1 contested, with reason, the use of the term calcification in the title as no

data of e.g. CaCO3 precipitation per unit of time is present. We agree on this although we observe clear dissolution on spirorbis as presented in the fixative example. We nonetheless removed calcification from the title.

Abstract:

Referee #1:

- "line 1-12 Too much background presented here, should be shorten".

We cancelled the lines 1-12.

Referee #3:

- "abstract line 17-20 does not hold and should be re-phrased."

"At a finer temporal resolution, the tubeworm . . .respiratory activities of the host alga on the carbonate system." Replaced by "Tubeworm recruits exhibited enhanced calcification of 40 % during irradiation hours compared to dark hours, possibly reflecting an acidification – modulating effect algal photosynthesis as opposed to an acidification-enhancing effect of algal respiration"

Introduction:

All three referees have pointed the need for refocusing of this part proposing different orientations. We cancelled the first 10 lines, shortened the lines 16 to 24 and introduced nearshore and algal boundary layer carbonate system variability in two distincts paragraphs.

Referee #1:

-"P3741 line 2 H2O rather than CO2 is the substrate of gaseous oxygen in photosynthesis."

We agree, that was poorly formulated, this part (line 1-10) has been removed in the final MS.

-“ P3741-3742 the authors should address more about coastal ocean acidification. . .”.

We agree on the importance of this topic and we propose in the new MS more details about variations of the carbonate system in the Baltic Sea macrophyte meadows that are of interest here. “The Baltic Sea is prone to acidification due to its low salinity, alkalinity and temperature such that undersaturation for calcium carbonates (omega arag and omega calc < 1) occurs naturally in winter (Thomas and Schneider, 1999; Tyrell et al., 2008). In spring and summer, the photosynthetic and respiration activities of phytoplankton and benthic macrophytes are imposing to the nearshore day / night variations of pCO2 of 200 to 400 μatm, causing fluctuation of omega from 1.5 to 2.5 (Thomas and Schneider, 1999; Saderne et al., in prep). In September, the intensification of westerly winds together with the collapse of the thermocline leads to the upwellings of hypercapnic bottom waters to the nearshore habitats (Thomsen et al., 2010). During such events, daily means of pCO2 of 1600 μatm with night peaks up to 2600 μatm are measured in macrophyte stands (Saderne et al., in prep.). Thus, conditions in coastal habitats may be undersaturated for aragonite and calcite for several days despite the intermittent CO2 uptake by the macrophytes for photosynthesis.

Referee #2:

-“ no references or information is provided in the introduction to explain anything about previous work surrounding boundary layers (a huge field!) or formation around algae and implications for OA.”

That has been included in introduction in the re-shaping of the MS: “The epibiotic species spend at least the initial part of their life cycle within the diffusive boundary layer (DBL) surrounding the Fucus thallus. This layer typically is 50 μm to 1 mm thick depending of flow velocity (Wheeler, 1980; Hurd et al., 2011). It is characterized by the slow diffusion of molecules, creating steep concentration gradients of compounds produced or consumed by the alga and its micro- and macroepibionts (Wheeler, 1980; Koch, 1994; Stevens and Hurd 1997; Hurd, 2000, Wahl et al. 2012). Thus, as an ex-

ample, in the boundary layer on the thallus of Fucus vesiculosus pH may vary between 8 in the dark to 9.2 at 785 μE light (Spilling et al. 2010). Therefore, we would expect this space to shelter sessile calcifiers from ocean acidification during daylight hours (Hurd et al. 2011) but possibly enhance acidification stress during the night.

Referee #3:

The referee #3 assessed fair critics about the hypothesis tested by the experiment on spirorbis juveniles.

We agree that the method employed do not allow assessing a clear link of causality between pH in the boundary layer and juvenile growth. Also the observed result can be indeed be simply caused by a reduced activity in the dark. Thus our assumptive interpretation as based on correlative observation. We have mitigated our statements in hypothesis and discussion in the new version of the MS. New MS hypothesis section: “In the present study, we tested the hypothesis whether ocean acidification (i) impacts epibiotic species and (ii) disadvantages the calcifying sessile fauna of Fucus serratus over the non-calcifying one. The calcifying tubeworm Spirorbis spirorbis and two bryozoan species, the calcifying Cheilostome Electra pilosa and the keratinous ctenostome Alcyonidium hirsutum were investigated regarding their growth, and recruitment (Spirorbis only) during 30 days of incubation under three pCO2 conditions. In addition, we tested at the same pCO2 conditions the effect of light on the growth of the Spirorbis recruits as a first indicator of a potential protection of calcification in the DBL by the host algae metabolism.”

Material and methods: We rephrased and reorganized this part to simplify the comprehension of the experimental design.

Referee #1:

-“illustrative image (Fig 1), which is not strong enough to convince reader that shell was corroded under high CO2/low pH.” We disagree here. While we did not accurately

quantify decalcification, the shell does look clearly corroded on a least 30 % of its surface and the worm soft body is exposed to seawater.

-"And what is the accuracy of epifluorescence microscope to detect calcification differences among CO2 treatments?"

We did not quantify calcification rates under the microscope but the growth of skeletal elements (tube, cystids). For this purpose, we took photo in high resolution of part of the colonies and assembled them to obtain a single picture of the whole colony of a size up to 50 000 x 50 000 with a resolution of 1 pixel approx. equal to 0.04 μm2. So that instrumental error is extremely small. We hope that this answer to be satisfying...

-the first referee asked the very interesting question of potential variation of growth over time and why, to tackle this, we did not sampled every 5 days instead of at the beginning and at the end of the incubation.

Our low resolution data are of course weaker than a more frequent sampling. However, the analysis method used being destructive, a more frequent sampling would have severely disturbed the experiment and complexify the statistical analysis. Non-linear growth is very characteristic of batch culture of bacteria or planktonic species but should not exist with our metazoans over 30 day of experiment. First of all, we considered the non-linear area increase of bryozoan by the use of logarithmic relative growth rates. Animals were fed every 3 days after water change with microalgae. Reduced growth due to age related senescence is not relevant in the case of bryozoans. There is a turnover of zooids within the colony making the age, as the time spent since the settlement of the larvae who created the first zooid, an irrelevant concept so that bryozoans are often called immortal (Nilsson Sköld and Obst, 2011). The worms were all adults of approx. the same size and then age, we have no reason to assume senescence to have happened in our experiment. The growth patterns of the tubes are detailed by Daly (1978), the SD was less important than for the bryozoan, proving that the method worked quite properly.

Referee #2:

-"How many replicates for each treatment were there and where they independent (a prerequisite for ANOVA). It could be from the way it is explained that there was one flask per treatment."

There was not 1 but 10 replicates per treatment. One replicate was 1 piece of fucus bearing all three epibionts together in its own flask of 600 mL. The prerequisite for ANOVA was therefore respected.

-"How stable was pH in the culture containers?"

The stability of the pH over the 30 days in the culture flasks are represented in the Table 1. Every 3 days, 3 of the flasks were sampled for carbonate chemistry in each treatment. We averaged those three to obtain one single value per treatment. The data we presented in table 1 is the mean $\pm$ SD of the carbonate system over 30 days. So, the SD during the 30 days was 0.031, 0.076 and 0.079 pH units in the control, 1200 and 3150 μatm treatment respectively. We clarified this part in the MS.

- "Was the correct pCO2 bubbled into flasks continuously or water changes made every three days?"

The correct pCO2 was constantly bubbled in every flask so that there is no pCO2 changes linked to outgassing or biological activity in the flasks. We clarified this part in the MS.

- "pH could not have remained consistent with 10,000 cells ml-1 of Rhodomonas, seaweed tissue and epifauna over three days in a 650 ml flask"

The only thing that has affected the pH was the variation of alkalinity due to calcification and dissolution. The alkalinity presented in Table 1 was measured after periods of 3 days so that the change of alkalinity between the treatments can (merely) only be associated to calcification and dissolution, photosynthesis and respiration are almost neutral to alkalinity (Zeebe and Wolf-Gladrow, 2001). The addition of rhodomonas

changed the alkalinity as we were adding the Provasoli's medium with the microalgae. This change was nevertheless common to all flasks as the same volume was added. Anyway, again, we were forcing the pCO2 constantly by bubbling so that this was absolutely constant (or at least not depending of the biological activity in the flasks). -"Water motion. Was there any in the culture containers, what were the likely size of boundary layers. This could be determined from the literature but It would probably be ideal if you measured them"

There was an important water motion in the flask due to the vigorous bubbling. The movement was therefore not laminar but highly turbulent due to the unequal shape of the Fucus sections. Making any evaluation or measure of the boundary layer was impossible. Nevertheless we have made our possible to insure a similar water motion in each flask by placing the bubbling stones in the exact same place and equalizing the air flow. We are discussing further this item in the revised MS.

Referee #3:

-"The term acclimation is arbitrary"

Acclimation was omitted in the new MS and replaced by "Staining" as suggested.

-" how do authors know that 7 days acclimation is sufficient and then did they allow for "acclimation" after transferring to high pCO2?"

As pointed out previously, the 7 days of acclimation were actually of preparation. Epibionts of brown algae are used to important variations of abiotic parameters because of the versatility of their habitat. Five day was in fact the necessary time to surely stain all the Spirorbis and Electra. Extending this period would have been useless and problematic for the experiment: the animals fed ad libitum were growing quite fast and would have overgrown the fucus pieces.

-"cutting of fucus thallii and 5-day staining – within this 7-day "acclimation?"

No, the cutting was made two days after collection in the field.

-"The carbonate chemistry should have been determined in the incubations for different epibionts and mixing tanks separately and not as a mixed "randomized" replicate. It is likely that changes during the 3-days take place in the seawater carbonate chemistry and this would have been interesting to document according to epibiont taxa studied. As 3 pCO2 levels are very different this might not be very important, but it would be good however, to demonstrate how variable the pCO2 levels were within the 3 "replicates" nevertheless."

In each replicate, all the epibionts were together on the same Fucus section, in one single bottle. No possibility therefore to assess the effect of each epibiontic taxa independently on the carbonate system. The averages of the standard deviations between the three flasks along the 30 days incubations for the pCO2 were of 11, 13 and 9 % in the control, 1200 and 3150 μatm treatments. We observed a significant increase of alkalinity after 3 days periods in the 3150 μatm treatment compared to the two others, reflecting the dissolution of the skeletal parts of the epibiontic community. However, this result was not presented as the change due to calcification / dissolution was confounded with the change due to the Rhodomonas medium.

Results:

In the new version of the MS, we omitted the data already presented in the graphs. Referee #2

- Shouldn't standard error be used in these figures instead of standard deviation? We changed in the figures SD for SE.

Referee #3:

- p. 3749 line 21f – what means "marginally affected"! Is that really so –> authors should critically consider their finding, that in E. pilosa the ambient is neither significantly different from either 1200 or 3150 μatm, while due to the fact, that due to a slightly more pronounced difference between the 1200 and the 3150 μatm there is a

significance – the question would be justified if here the statistical result needs to be considered with caution?

The reason for this statement was not only based on this statistical difference but also on the observed dissolution of the non-occupied cystids (bryozoan box like skeleton). However, we replaced “marginally affected” by “unaffected” in the new version of the MS.

- It would have been interesting to test a “species effect” if data between species can be normalized accordingly as this addresses the main hypothesis.

Normalization problem was indeed the reason why we haven’t done so. Relative area growth rates were considered for the bryozoan while for Spirorbis length increments. It was not possible to produce a common valid unit of growth to make comparisons.

- The figures are well illustrating results, specifically by including the statistical results. Note, that in Figure 2B the significance level indicated by the asteriks cannot be correct as the one with bigger difference has a lower significance level?

Thanks, indeed there was a mistake. . .

Discussion:

We rewrote this part by giving less space to calcification data and more to the effect of boundary layer, as requested by the reviewers. More specifically, the referee #3 wrote “If discussing any likely effect related to carbonate chemistry in the boundary layer, the authors should at least try to give an estimate pH range that may be expected and consequently of other parameters of the carbonate chemistry at each of the three pCO2 levels if they want to address this issue”. It is impossible to give estimate from the literature of the pH at the three pCO2 in the boundary layer. Giving two other parameters is even more impossible as the carbonate chemistry in a boundary layer is not a state of fully relaxed equilibrium, so that the usual way to derivate the carbonate system from two parameter is non-valid (Zeebe et al., 1999). Each parameter has to be

measured independently or indirectly calculated from complex kinetic models. So for example, to calculate the saturation state of CaCO3 in a boundary layer it is necessary to use CO32- microsensors (assuming the [Ca2+] constant). This technology exists but is not purchasable (Beer et al., 2008; De Beer, pers. comm.) and anyway we would go back to the problem of the non-laminarity of the flow in our experiment flasks. We discuss both the flow issue and the complexity of the boundary layer carbonate system in a new version of the MS.

References:

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