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Interactive comment on "Intercomparison of four methods to estimate coral calcification under various environmental conditions" *by* Miguel Gómez Batista et al.

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We thank the reviewer for her/his comments and suggestions on our manuscript. We agree with most comments and modified/updated the manuscript accordingly. Below is a point-by-point reply.

This is a nice study comparing 4 different methods to measure short-term calcification rates in corals. The comparison of three less commonly used methods (calcium anomaly, 45Ca, 13C) with the commonly used alkalinity anomaly technique adds to the existing literature of method comparisons for estimating coral calcification. Furthermore, the current study has the benefit that the different methods were measured

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during the same incubation, minimizing the risk of other factors confounding the results. The authors show that two of the three methods are highly correlated and not significantly different from the alkalinity anomaly technique, and further provide useful recommendations on minimum and maximum incubation times for various volume to biomass ratios and techniques. Overall, this will be a useful addition to the existing literature on coral calcification methods. As a note of caution, I do not have experience with the calcium anomaly, 45Ca and 13C methods, therefore I cannot judge the experimental protocol used for these methods.

I only have one concern regarding the data: since there was no pH control during the incubations and some incubation times were rather long, especially when conducted in the dark, significant changes in carbonate chemistry did occur over the course of these incubations. For example, pH decreased from 8.05 to 7.62 under ambient conditions in the dark due to respiration and calcification. While this is clearly stated in the Results, the Discussion on acceptable changes in carbonate chemistry largely focuses on changes in delta CT rather than pH but I don't think such a change is acceptable in studies that actually aim to detect the impacts of low pH on coral calcification. Similarly, Riebesell et al. (2010) also recommend that changes in AT during incubations should be within 10% of starting AT, yet changes in this study were typically larger than this, except under low pH. Furthermore, there is no discussion whatsoever regarding changes in dissolved oxygen and this was also not measured, despite hypoxia/hyperoxia potentially stressing the corals. Again, while this may be less relevant for a method comparison, it is certainly relevant when making recommendations for general incubation times. I would therefore encourage the authors to discuss these aspects in more detail in the Discussion.

Many thanks for these very constructive comments. As stated in the manuscript (but clarified in the revised version), our study was designed to compare different techniques to estimate calcification rates and not to define the best experimental approach to study the effects of ocean acidification on coral species using these different tech-

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niques. As such, the chosen experimental protocol (e.g. incubation times) was not optimal and led, in some cases, to significant changes in the carbonate chemistry during incubations. We fully agree with the reviewer that the method we used to estimate maximal incubation times (i.e. only implying a change in CT < 10%) is not acceptable. Indeed, as stated by the reviewer, one should not only focus on CT but on pH and AT as well in order to make sure that carbonate chemistry is maintained under an acceptable range (as compared to starting conditions). While we could find in the literature some estimates of "acceptable" changes in CT and AT (respectively Langdon et al., 2010 and Riebesell et al., 2010), it is more difficult to estimate what changes in pH are acceptable. As such, we have arbitrarily decided to consider a maximal change in pH set to 0.06 which is the minimal change in global surface ocean pH projected for 2100. Therefore, the new estimated tmax corresponds to the lowest value between tmax_pH (DpHT < 0.06), tmax_CT (DCT < 10%) and tmax_AT (DAT < 10%). Except in the light under ambient pH conditions, tmax is always set to the maximal incubation time allowed to keep pH levels under an acceptable range (DpHT < 0.06).

Regarding oxygen levels, as pointed out by the reviewer, oxygen levels were not measured. However, our incubations were conducted in continuously mixed open systems, allowing equilibration with the atmosphere. Exchange at the air-sea interface is considerably faster for O2 than for CO2. Furthermore, we have unpublished data from an other experiment that confirm that under the same experimental setup, where we also tracked the dissolved oxygen concentration over time, we did not observe any significant deviation from saturation.

The new paragraph now reads: "Our study was designed to compare different techniques to estimate calcification rates and not to define the best experimental approach to study the effects of ocean acidification on coral species using these different approaches. As such, the chosen experimental protocol (e.g. incubation times) was not optimal and led, in some cases, to significant changes in the carbonate chemistry during incubations. However, our results provide some insights that we further discuss in

the following section. Measuring and comparing calcification rates of organisms under varying pH conditions requires the careful choice of a volume and a time interval such that the precision of the calcification rate measurement is large enough to observe significant signals and that the change in carbonate chemistry parameters between the beginning and end of the incubation is small compared to the range of these parameters in the different treatments (Langdon et al. 2010). Table 5 illustrates the incubation time necessary to obtain measurable changes for each method (tmin) considering the ratio between incubation volume and coral size chosen for our study. As the 13C incorporation method did not provide reliable rates, this technique was not considered in this analysis. The threshold for significant signals was set at 10-fold the analytical precision of the instruments (Langdon et al. 2010) for AT and Ca2+ measurements (1.2 and 2.9 μmol kg-1, respectively) and above the detection limit of 15 cpm for 45Ca activity estimated. Maximum incubation times are more difficult to estimate. Langdon et al. (2010) and Riebesell et al. (2010) recommend considering incubation times short enough to maintain AT and CT within an acceptable range (ïĄĎAT and ïĄĎCT < 10%). As it is more difficult to estimate what changes in pH are acceptable, we have arbitrarily considered a maximal change in pH of 0.06, corresponding to the lowest change in global surface ocean pH projected for 2100 (IPCC, 2014). Maximal incubation times, as presented in Table 5 (tmax), correspond then to incubation times that should not be exceeded in order to maintain acceptable conditions of the carbonate chemistry (DpHT < 0.06 and DAT < 10% and DCT < 10%). Under light and ambient pH conditions, even if the ratio between incubation volume and nubbin size is much higher than for previous similar studies (e.g. Cohen et al. 2017), all methods would allow a precise estimation of calcification rates over very short incubation times ($\sim$15 min to 1 h, depending on the method) while leading to moderate changes in carbonate chemistry. In the dark, and under ambient pH conditions, in the absence of pH increase due to photosynthesis, the decrease of pH due to respiration, narrows the possible incubation period to 1.3 h. While this is still larger than the incubation time allowing to obtain a significant signal with alkalinity anomaly technique ($\sim$20 min), the other two methods necessitate longer

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incubation times to obtain precise estimates (> 1.5 h). At lower pH, both under light and dark conditions, and using open systems without a continuous pH regulation as in our study, it is obvious that all techniques are not well adapted to this experimental protocol. Indeed, as a consequence of lower calcification rates at lower pH and significant CO2 degassing, incubation times necessary to obtain significant signals using these techniques are too large to maintain the carbonate parameters within an acceptable range. This is not insurmountable as a continuous regulation of pH using for instance pure CO2 bubbling or incubations performed in a closed container (i.e. without contact to the atmosphere) would alleviate these problems.

Specific Comments

Abstract

L32: please state the respective pH values instead of ambient and low

Added

Introduction

L61: please also cite here other studies that recently compared various calcification methods, such as (Gazeau et al. 2015), (Schoepf et al. 2016) and (Cohen et al. 2017)

References added.

L84: “solid agreement” – this is rather colloquial and should be rephrased, e.g. “good agreement”

Modified

L114: you could add here that this was done under different pH and light conditions

Added

Methods

L124-138: Please provide more information on how water motion/flow was provided in

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the aquaria, how big the tanks were, rate of seawater renewal etc

Now provided.

L127: please provide more information on how many branches from how many different parent colonies were collected for each experiment.

It now reads: “In June 2017, 40 terminal portions branches of S. pistillata, free of boring organisms, were cut from four different parent colonies (10 branches per parent colony) and suspended by nylon lines to allow tissues to fully cover the exposed skeleton for at least five weeks (Tambutté et al., 1995; Houlbrèque et al., 2015).”

L130: what was the concentration of Artemia fed during experiment 1? This info is only provided for experiment 2

Added.

L137: please change to “biometrics parameters of the biological material”

Modified.

L146: looking at Fig. 1, I wonder whether the rod to which the nylon line was attached shaded the coral from light coming from above?

The thickness of the holder was only 4 mm. The position of the lights and water movement inside the incubation chamber allowed nubbins to slowly move inside the chamber and ensured no significant shading.

L147: should be “and low pH”

Corrected.

L273: a description of how coral skeletal dry weight was measured is missing from the Methods. Please add.

This was mentioned in the text, we added the apparatus used to weigh the samples: “Tissues were then dissolved completely in 1 mol L-1 NaOH at 90 °C for 20 min. The

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skeleton was rinsed twice in 1 mL NaOH and twice in 5 mL in MilliQ water. It was then dried for 72 h at 60 °C, precisely weighed at +- 0.01 g using a Sartorius BP 310S (referred thereafter to as skeleton dry weight), and dissolved in 12 N HCl."

L309: It's good to see that model II regressions were used for the analyses.

Thanks, this is indeed appropriate when both variables are associated to experimental errors.

Results

L313: Table 2: why was the seawater activity much higher in experiment 2 than 1?

As we anticipated calcification rates to be lower at lower pH, it was decided to double the activity in seawater. This is now clarified in the text.

L316: please state whether this is SD or SE

Since we present SD values for all environmental conditions (as opposed to SE when we refer to estimated rates), a sentence has been added at the start of the Results section: "All values in Table 2 as well as in the text below correspond to the average between replicates (or incubations) ± standard deviation (SD)."

L328: was this change in pH during incubation similar for the different methods?

Indeed, as mentioned in the text, changes in pH were similar for the different incubations. Final pH levels were:

In the light

45Ca: pHT (8.05 ± 0.03; n = 6)

13C: pHT (8.06 ± 0.04; n = 6)

In the dark

45Ca: pHT (7.61± 0.1; n = 6)

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13C: pHT (7.63 $\pm$ 0.04; n = 6)

L336: should be “were similar”

Corrected

L361: there are also some other data with asterisks in Table 3 – I assume they are also outliers but this is not explicitly discussed. Please clarify.

Clarified: “These estimates (n = 4) have been considered as outliers, marked with an asterisk in Table 3 and not included in the following analyses.”

Discussion

L443: please replace “that” with “why”

Replaced.

L461: should be “was” x2

Modified.

L492: should be “importantly”

Modified to “significant”.

L514: would be necessary for what? Please add.

Modified to: “Conducting similar comparison studies with other coral species as well as other major calcifying groups widely studied in the context of ocean acidification (e.g. coralline algae, molluscs etc. . .) would be necessary for a better understanding of ocean acidification impacts on ecosystem services provided by calcifying organisms.”

Figures and Tables

Table 3 is very long. I think this information could be better represented in a figure showing both the average of all six replicates per treatment/method and the individual data points spread around the average.

We respectfully disagree and prefer keeping the table as it is, as we believe it is important to provide the actual numbers to the reader. Individual data points are further shown in Figures 2-4.

Also, the legend does not currently explain what the asterisk next to some data means. Please add.

Added.

Table 4: please add the p-value for the regressions to the table.

Added.

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