

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

### **Anonymous Referee #2**

Received and published: 14 November 2019

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,  $^{45}\text{Ca}$ ,  $^{13}\text{C}$ ) 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 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

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literature on coral calcification methods. As a note of caution, I do not have experience with the calcium anomaly,  $^{45}\text{Ca}$  and  $^{13}\text{C}$  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.

### Specific Comments

#### Abstract

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

#### 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)

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

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agreement”

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

#### Methods

L124-138: Please provide more information on how water motion/flow was provided in the aquaria, how big the tanks were, rate of seawater renewal etc

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

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

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

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?

L147: should be “and low pH”

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

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

#### Results

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

L316: please state whether this is SD or SE

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

L336: should be “were similar”

L361: there are also some other data with asterisks in Table 3 – I assume they are also

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outliers but this is not explicitly discussed. Please clarify.

#### Discussion

L443: please replace “that” with “why”

L461: should be “was” x2

L492: should be “importantly”

L514: would be necessary for what? Please add.

#### 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.

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

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

#### References

Cohen S, Krueger T, Fine M (2017) Measuring coral calcification under ocean acidification: methodological considerations for the <sup>45</sup>Ca-uptake and total alkalinity anomaly technique. *PeerJ* 5:e3749

Gazeau F, Urbini L, Cox TE, Alliouane S, Gattuso JP (2015) Comparison of the alkalinity and calcium anomaly techniques to estimate rates of net calcification. *Mar Ecol Prog Ser* 527:1–12

Riebesell U, Fabry VJ, Hansson L, Gattuso JP (2010) Guide to best practices for ocean acidification research and data reporting. European Commission Publications Office,

Schoepf V, Hu X, Holcomb M, Cai W-J, Li Q, Wang Y, Xu H, Warner ME, Melman

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TF, Hoadley KD, Pettay DT, Matsui Y, Baumann JH, Grotoli AG (2016) Coral calcification under environmental change: a direct comparison of the alkalinity anomaly and buoyant weight techniques. *Coral Reefs* 1–13

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