

**Dear Editor,**

We would like to thank Reviewer #1 for his/her thoughtful and detailed comments on our manuscript. We accepted and dealt with most of the comments and changed the MS accordingly.

Below are the point-by-point replies to comments and suggestions made by the reviewer.

**“The incubation for TA without prior exposure time to reduced pH was carried out in an open system while the other experiments were carried out in a closed system, and the "reduced" initial pH-level was 7.19 in the open system (with an  $\omega < 1$ ), while in the closed system it was 7.49 ( $\omega > 1$ ). The closed system approach resulted in a drastic change of the carbonate chemistry (as shown in Table S3). Specifically the  $p\text{CO}_2$ -level should have been maintained constant during incubation (for example by bubbling). These drastic changes led to rather uncontrolled conditions with respect to the carbonate chemistry and render any interpretation with respect to OA greatly ambiguous. Here definitely an open system with  $p\text{CO}_2$  bubbling would have been required”.**

REPLY: Indeed, “open” systems are more desirable when dealing with organisms that may alter the chemistry of the surrounding seawater while calcifying, photosynthesizing or respiring.  $\text{CO}_2$  bubbling is a valid method to maintain a constant pH during perturbation experiments (provided that  $\text{CO}_2$  dissolves no slower than uptake of  $\text{CO}_2$  by biological activities), however, at steady state, this should not affect and definitely does not compensate for the changes in TA. Moreover, evaporation of seawater from the incubation vessels may increase the alkalinity due to change in salinity. In general, this type of setup using  $\text{CO}_2$  bubbling are more complex and time consuming (see Jury et al. 2010), resulting in a smaller number of repeats. It becomes even more complex when using radioisotopes to measure calcification.

The drastic changes in carbonate chemistry the reviewer refers to occurred after 4 and 6 h incubation, respectively, in reduced (7.49) pH treatment (referring to the results for the corals that were pre-exposed for 14 months to the low pH treatment). There are no solid rules regarding the percentage changes allowed to occur during perturbation experiments. Yet, while the OA community is still debating on how changes in the seawater carbonate chemistry may affect coral physiology, in particular coral

calcification, it is recommended that changes in TA and DIC should not exceed 10% (Schulzs et al., 2009; Jury et al., 2010; Riebesell et al., 2010). Consequently, and as explained in the MS, we only analyzed the NC and GC rates after 2 h of incubation where changes in TA and DIC were still within the acceptable range. In the current study, shifts in pH (from its initial values) during the incubation period (Table 3; referring to the results after 2 h of incubation for the corals that were pre-exposed for 14 months to low pH treatment) ranged between 1-4% (results not shown) regardless of the pH treatments (7.19, 7.49 and 8.09), illumination conditions (light and dark incubation) or open/closed vessels. Nevertheless, as there are only recommendation for TA and DIC variations (Schulzs et al., 2009; Jury et al., 2010; Riebesell et al., 2010), we suggested in the MS that one should take into consideration the variations in the other components of carbonate chemistry as well, i.e.  $\text{CO}_3^{2-}$  that occur together with the variations in TA and DIC (as outlines in the MS p. 8255 line 14), particularly in  $\text{CO}_2$ -enriched seawater which has a lower buffering capacity and may result in a stronger drift in  $p\text{CO}_2$  and pH in response to biological activity (Suzuki 1998; Delille et al. 2005; Riebesell et al. 2007). Our study employed standard approaches for conducting the incubation experiments to allow comparison of our data set with previous studies, but at the same time we highlight some uncertainties in those 'acceptable' ranges that may be informative and valuable to the OA community.

**The manuscript is confusing as it is hard to follow with respect of experimental design (net, gross calcification, light/dark incubations etc., dissolution of bare skeleton and which reduced pH level 7.19 or 7.49, and open and closed system).**

REPLY: We added to Table 3 a column indicating the illumination conditions (light/dark) during the short-term incubation and noted in which experiments we measured the dissolution of bare coral skeleton:

Table 3. Details of experimental set-up. O, open; C, closed; NC, net calcification; GC, gross calcification. Dissolution of bare coral skeleton was measured in corals that were not acclimated/pretreated with reduced pH prior to incubation ( $\text{pH}_T$  7.19) and corals that were pretreated for five months prior to incubation ( $\text{pH}_T$  7.49).

Pre-incubation		Incubation experiment						
Exposure period to pH treatment (months)	Open/Closed vessels	Water motion	Fragment size (cm <sup>2</sup> ) /volume ratio (ml)	Incubation period (h)	Initial pH <sub>T</sub>	Repeats	Measurement type	Illumination
No pre-inc.	O		0.35-0.45	3	8.09 & 7.19	7	NC	Light and dark
2	C	×	0.14-0.18	1	8.09 & 7.49	5	NC	Light and dark
5	C	×	0.07-0.1	1	8.09 & 7.49	9	NC	Light and dark
14	C	×	0.14-0.18	2, 4 and 6	8.09 & 7.49	6	NC and GC	Light

**p. 8243, line 18: "true" calcification should be deleted - net and gross calcification can clearly be defined and what in the end would be the "true" calcification is a matter of debate as one may also state, that only the calcium accreted after a certain time is the "true" calcification and this would be "net".**

REPLY: The term "true" calcification was simply used to define calcification rate before any deductions for dissolution were made. This term was already used in Riebesell et al. (2010) to distinguish gross calcification from net calcification. We have changed this sentence to read: "...is due primarily to a decrease in the "true" calcification (calcification rate before any deductions for dissolution have been made equals the gross calcification; Riebesell et al. 2010), increased skeleton dissolution (gross dissolution) or a combination of both (net calcification; Rodolfo-Metalpa et al., 2011)." In the text we will cite this reference each time that the term "true" is utilized.

**p. 8244, line 18 ..."only the oral ectoderm is in direct contact with seawater" I doubt this is true, seawater can access via the mouth and coelenteric to the endodermal tissue. Then there is some believe that the calciblastic layer might be replenished (from time to time) with seawater... this does still not contradict, that the tissue layer form a barrier where chemical gradients can be obtained between calciblast and ambient seawater.**

REPLY: We agree with this comment. We changed the sentence as follows: Of the four single cell thick epithelial layers (oral ectoderm and endoderm and the aboral ectoderm and endoderm) interconnected by the thin non-cellular mesoglea, the oral ectoderm and endoderm are in closest contact to seawater (Gatusso et al. 1999; Kleypas et al. 2006).

**p. 8247 chapter 2.2.4 – this is unclear. authors should be more specific – what time of day and was there light and dark incubations carried out – may be Table 3 can be complemented with respect to the repeated measurements being carried out with a same specimens**

REPLY: We agree with this suggestion. Both experiments, comparing gross and net calcification, were conducted under light conditions and, as explained above, we added to Table 3 a column describing the illumination conditions in all the experiments.

**p. 8248, - line 11 – NBS scale is “out-dated” and total scale should have been measured.**

REPLY: We agree that pH measurements on the NBS scale are less precise for calculating carbonate system species in seawater due to the variation in ionic strength between buffers and the seawater (Dickson, 1984; Millero, 1986; Zeebe and Wolf-Gladrow 2001). Preparation of synthetic seawater for total scale measurements with the high ionic strength of the Red Sea (~0.85 compare to ~ 0.7 of oceans; Zeebe and Wolf-Gladrow 2001), however, is very complex. In addition, our pH system were calibrated over several years with the NBS scale, before NBS were "out-dated", and thus we must use the same calibration method throughout the experiments (Riebesell et al. 2010). Given these difficulties, the  $pH_{NBS}$  were shifted onto the total pH scale ( $pH_T$ ) by subtracting  $-0.11$  (Zeebe and Wolf-Gladrow, 2001). It should be noted that the relative differences between control and treatment are an order of magnitude different and the lack of measurements in pH total scale (based on artificial seawater) had little effect (Andersson et al. 2009).

**p.8248, - line 25 - instead of “numerous times” authors should give number or time interval for measuring**

REPLY: We agree with this comment. We changed the sentence: "Seawater samples from the pH system were measured for total alkalinity to monitor the system 5-8 times over the experimental period ( $2501 \pm 13$ ,  $2491 \pm 9$  and  $2501 \pm 6$   $\mu eq\ k^{-1}$  for  $pH_T$  7.19, 7.49 and 8.09, respectively; Fig. S1)".

**Results 3.1: as pointed out in my major concern, the comparison of net calcification between non-exposed and exposed to reduced pH prior to incubation is not valid because two systems (open-closed and two different pH levels were used). Why the carbonate chemistry for dark incubations is not given? This is important, and if data are available, this could “save” the MS and help for interpretation of results**

REPLY: See our response to this in the first paragraph.

**Results 3.2, line 17 what is meant by 1:1 regression slope – a linear regression ?**

REPLY: A 1:1 regression slope defined as a regression slope when  $x=y$ . We changed the sentence to clarify it: "The relationship at low pH treatment was less correlated ( $r = 0.561$ ,  $p = 0.015$ ) and did not follow the 1:1 ( $x=y$ ) regression line as indicated by the regression slope."

**Discussion 4.1. this discusses the obvious and is not related to author’s findings as they state themselves in the last sentence**

REPLY: Our results of NC with the dissolution rates of bare coral skeleton were discussed in this paragraph and compared with the Rodolfo-Metalapa et al. (2011) findings from temperate scleractinian corals. To the best of our knowledge, this is the first time that such a comparison between NC and dissolution rates was conducted for *Stylophora pistillata*, a tropical scleractinian coral, thus it may yield valuable information regarding tropical corals ability to maintain positive calcification under OA conditions. The last sentence in this paragraph stated that measuring NC gives us half information as we cannot distinguish between the real calcification ability and dissolution if it occurs, for example when positive net calcification values are obtained. Therefore, gross calcification was also measured in this study for the corals that were exposed to the reduced pH treatment 14 months before performing the incubation experiment. We changed the last sentence to clarify this point: "In this kind of experiment, however, we cannot distinguish between the true rate of calcification and dissolution because alkalinity measures net values only. In an attempt to separate these two processes and determine their respective roles in affecting coral calcification under the predicted global changes, we measured gross calcification as well, for corals that were pre-exposed to the reduced pH treatment 14 months before performing the incubation experiment."

**Discussion 4.3.1- The first paragraph could be more specific/quantitative and related to the results the second paragraph discusses the major problem of using a closed system approach, the last sentence is superfluous – it is not that more intercomparison studies are needed, but that they are carried out with the appropriate approach and analyses.**

REPLY: We changed the last sentence of the paragraph: "Additional intercomparison studies are therefore needed to test the compatibility of the total alkalinity and  $^{45}\text{Ca}$  techniques for OA research while choosing the appropriate volume and incubation time to avoid substantial shifts in the carbonate chemistry during the incubation period".

**Discussion 4.3.1- Authors discuss at length some mechanistic explanations why results are variable, however, they do not discuss, that interestingly, the TA method appears to provide consistently higher calcification results than the  $^{45}\text{Ca}$  (although not significant) and what this means with respect to determination of “net” and “gross” calcification – f.ex. the TA could have been influenced by inorganic nutrient uptake during photosynthesis (I guess nutrients were not determined from incubations). Adversely, if dissolution takes place, newly incorporated  $^{45}\text{Ca}$  would also dissolve again, meaning, that the  $^{45}\text{Ca}$  method does not measure gross calcification either, but - at most - has less dissolution effects (as also older skeletal parts prior to labelling dissolve).**

REPLY: In section 4.3.2, line 23, of the discussion we do mention that the means of NC rates were higher than GC rates (although not significant). However, as was mentioned previously, we only refer to the results obtained after 2 h of incubation. Inorganic nutrient uptake during the photosynthesis process by the coral's symbionts cannot be the reason for the higher NC rates observed. Chisholm and Gattuso (1991) validated the use of the alkalinity anomaly technique for the determination of coral calcification rates during short-term (12 h) incubation without further correction for changes in nutrient concentration (the nutrient corrections which apply are smaller than the variability shown in calcification data). Thus, it is unlikely that NC rates should be higher than GC rates, particularly under low pH treatment. As for dissolution of newly incorporated  $^{45}\text{Ca}$ , it is well accepted that the  $^{45}\text{Ca}$ -labeled technique is likely to provide measurements of gross calcification when conducted over short-term incubation (Kleypas et al., 2006; Riebesell et al., 2010)

**The conclusion with respect to “acclimation” is not valid with the experimental results and flaws pointed out above.**

REPLY: We disagree with the referee on this point. Acclimation is a valid point to discuss in this case even if we did not prove that it occurs. The OA community is increasingly interested in acclamatory processes, hence we feel this is an important issue to discuss. This in turn may stimulate and give rise to future studies.