## Dear Editor,

We would like to thank Reviewer #2 for his/her thoughtful and detailed comments on our manuscript. We dealt with and accepted most of the comments and changed the MS accordingly.

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

To distinguish gross and net calcification, the authors used TA and 45Ca methods. However, if dissolution occurs repeatedly, 45Ca method is not always valid for measuring gross calcification (e.g., if coral skeleton including 45Ca dissolved into seawater).

<u>REPLY</u>: It is well accepted that the <sup>45</sup>Ca-labeled technique is likely to provide direct measurements of gross calcification when conducted over short-term incubation (Kleypas et al., 2006; Riebesell et al., 2010). Furthermore, it is implausible that live microcolonies (completely covered by tissue) dissolved at the low (7.49) pH treatment as dissolution was not detected for bare coral skeleton at the same conditions.

I could not understand why the authors performed long-term experiment although the authors emphasized the importance to distinguish between gross and net calcification. Was long-term experiment necessary for the authors' main aim? I think the possibility of acclimation of corals to OA is interesting, but this would lead to many confusions in this manuscript.

<u>REPLY</u>: Long-term was used in the sense of acclimation prior to the incubation only. This is required to avoid any stress responses such as cessation of calcification. We do not see how this relatively long acclimation can confuse the reader or jeopardizes fulfilling the aims of this study; on the contrary, there is added value in it.

In Table S3, the values of the carbonate chemistry seem to be very unstable, which affected the result of coral calcification in this study. Thus, I am not sure whether the results presented here are valid.

<u>REPLY</u>: See our response to this comment in our reply to referee 1#.

Although the authors concluded that "S. pistillata may fall into the "low sensitivity" group", but previous studies suggest that this species is also affected by acidified seawater. This point should be discussed more carefully.

<u>REPLY:</u> Our result is consistent with the recent study of Houlbréque et al. (2012) showing that there was no change in the gross calcification rates of *S. pistillata* between the different pH (8.1, 7.8 and 7.5) conditions. Reynaud et al. (2003) as well did not observe significant changes in the calcification rates of *S.pistillata* under high (734  $\mu$ atm) *p*CO<sub>2</sub> treatment at 25°C compared to a normal *p*CO<sub>2</sub> treatment. The negative response observed in *S. pistillata* in previous studies (Gattuso et al. 1998; Marubini et al. 2008) can be attributed to the differences in the manipulation of carbonate chemistry and the latter is well discussed in the last paragraph of section 4.2. Moreover, data compiled from studies that examined the effect of low pH on coral net calcification revealed that there are more corals that fall into the 'high sensitivity' group (40-83% reduction in calcification) in relation to ocean acidification, whenever acid/base addition is being used to achieve a desired pH compared to changes in *p*CO<sub>2</sub> levels (reviewed by Langdon and Atkinson 2005).

We added in both text and Table 1 a reference to the paper by Houlbréque et al. (2012). We also added the study by Langdon and Atkinson (2005) in the last paragraph as a reference: "Our findings indicate that *S. pistillata* will be able to acclimate and even maintain normal calcification rates in a high  $CO_2$  world even if dissolution will occur during night-time, which implies that *S. pistillata* may fall into the  $CO_2$ -tolerant group (0-18% reduction in calcification in response to high  $pCO_2$ ; reviewed by Langdon and Atkinson 2005)".

Introduction: The authors should also add some information on light-enhanced or dark repressed calcification of corals in Introduction (e.g., see Gattuso et al. 1999) because the authors compared calcification between light and dark conditions. Gattuso JP, Allemand D, Frankignoulle M (1999) Amer Zool 39:160-183

<u>REPLY</u>: Scleractinian corals exhibiting higher rates of calcification in the light than in the dark. This phenomenon has been well documented in many previous studies as light-enhanced calcification (e.g. Goreau 1959; Gattuso et al. 1999; Furla et al. 2000) and is commonly attributed to the photosynthesis process by zooxanthella. This however is not in the scope of the study and therefore we added only a short paragraph mentioning the phenomena and the relevant references (p. 8244, line 26).

## 2.1 Coral preparation and maintenance. The authors used eight colonies of their target species, but I could not understand how many fragments were prepared from these colonies. The authors should add the detail.

<u>REPLY</u>: We agree with this comment. We changed the `repeats` column so each number will represent the number of coral fragments that were taken from each mother colony.

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.	0		0.35-0.45	3	8.09 & 7.19	3+2+2	NC	Light and dark
2	С	×	0.14-0.18	1	8.09 & 7.49	3+2	NC	Light and dark
5	С	×	0.07-0.1	1	8.09 & 7.49	3+3+3	NC	Light and dark
14	С	×	0.14-0.18	2, 4 and 6	8.09 & 7.49	6**	NC and GC	Light

\* Each number represents the number of coral fragments taken from the same parent colony.

\*\* Coral fragments were taken from the same mother colony to reduce the error probability especially when measurements are conducted based on such sensitive methods for measuring calcification (Total alkalinity and <sup>45</sup>Ca-labeling techniques).

## I recommend that the authors add the information on the reason for setting temperature as 25 degrees centigrade.

<u>REPLY</u>: We changed the sentence as follows: p 8245, line 19: "Temperature was regulated to ~  $25^{\circ}$ C (summer mean temperature when calcification is at its peak) using a..."

The nutrient concentration significantly affects coral calcification under acidified seawater condition (see Chauvin et al. 2011). I recommend that the authors add the information on nutrient of the seawater used in the study. Chauvin, A., Denis, V., Cuet, P., 2011. Is the response of coral calcification to seawater acidification related to nutrient loading? Coral Reefs 30:911-923

<u>REPLY</u>: We are surprised to have this comment from the reviewer as it is well known that the Gulf of Aqaba is one of the most oligotrophic seas. The nutrient concentration is almost irrelevant here. We have stated the oligotrophic nature of the gulf in the text

but see no reason to add concentrations although this information is readily available to us through the National Monitoring Program of the Gulf of Eilat.

2.2.1 Incubation procedure Although the authors proposed "we use the term "acclimation" in this paper to indicate long-term incubation at a certain pH condition" in this paragraph, I think this content should be separated in another paragraph to avoid confusion (e.g., "Short-term incubation" and "Long-term incubation").

<u>REPLY</u>: We performed only short-term (up to 6 h) incubation in vessels for the measurements of calcification values. The "long-term incubation" was referring to long-term exposure to a certain pH conditions. We understand the confusion and therefore changed the sentence as follows: "To avoid confusion, we use the term 'acclimation' in this paper to indicate long-term exposure to a certain pH condition".

## 3.2 Comparing gross and net calcification "repeated measure ANOVA"->"repeated measures ANOVA"

<u>REPLY</u>: Thank you for the comment. We will change it in the text.

Fig.1 I recommend the colors (grey and yellow) of bars should be changed to each other (light (yellow bars) and dark (grey bars)).

<u>REPLY</u>: We agree with this suggestion. The colors in the graph changed accordingly.

Table 3 Here, the authors showed Open/closed vessels, but I could not understand the reason why the authors used these two types. The authors should explain this reason in the main text.

<u>REPLY</u>: We agree with this comment. We will add to the electronic supplementary material under "incubation procedure" the following paragraph:" "Open" systems are more desirable when dealing with organisms that may alter the chemistry of the surrounding seawater while calcifying, photosynthesizing or respiring.  $CO_2$  bubbling is a valid method to maintain a constant pH during perturbation experiments (provided that  $CO_2$  dissolves no slower than uptake of  $CO_2$  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  $CO_2$  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. In light of these difficulties and to avoid calculating evaporation from seawater when working in open system, all incubation experiments, following the first one, were conducted in closed vessels". We will refer to this paragraph within Table 3.