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Interactive comment on "Respiration of Mediterranean cold-water corals is not affected by ocean acidification as projected for the end of the century" *by* C. Maier et al.

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Authors Reply to Anonymous Referee #1 Interactive comment on "Respiration of Mediterranean cold-water corals is not affected by ocean acidification as projected for the end of the century" by C. Maier et al.

-> We would like to thank the reviewer for his/her thoughtful comments on our manuscript. Our reply to respective comments is given below and will be considered when revising the manuscript:

R1: "It is really a shame the Author did not include these data on the last recently published paper concerning the effects of acidification on Mediterranean cold water

corals (Maier et al 2013). Together these new measurements would have a major value than presented in a separate study."

-> In principal, we agree, that data measured in parallel should be presented in one paper. But, vice versa, it does not mean that a separate publication diminishes the value of any of the findings – rather it allows one to focus on aspects of different questions. We opted to publish our calcification and respiration results in two consecutive papers for various reasons: We used a repeated measures design for both calcification and respiration and two cold-water coral species. This is very complex design for either variable. The repeated measurements provided more than 5-10x the data set than those in most other studies addressing either respiration, calcification or both. For calcification, we had additional data (initial controls and additional BW data after 9 months, where no respiration was measured). Thus, for respiration we used the same corals for which we determined calcification – however, for technical reasons, fewer replicates and no initial determination (at ambient pCO2) nor after 9 months. This discrepancy in the two data sets would have made it difficult to follow. To have a consistent data set for both calcification and respiration, we would have needed to reduce the data on calcification measured to match the N and repeated measures of the Respiration. This would have meant a loss of information with respect to calcification. Therefore, we strongly preferred to publish our results that neither short- or long-term calcification rates did not change over a very long time period and to subsequently address (in the present paper) the question of whether the higher energy demand needed to up-regulate the calicoblast pH to maintain calcification rates constant over a large pCO2 gradient was reflected in higher respiration rates. Besides the technical point, to put all these questions in one paper, would have overloaded the discussion as the two data sets had different foci with respect to the questions addressed. So in this case, we feel that separating the results into two papers rather increased the "value" as it allowed to address different questions and to re-combine aspects of calcification from the previous publication and respiration in the discussion of the present paper.

R1: "Actually, although the respiration rates of these corals have never been measured under acidified conditions, as several times repeated through the ms, this paper does not add new relevant insights."

-> We are not sure that we understand the reviewer. We have made novel measurements. If something has never been measured/published under certain conditions, how is it possible that it does not add relevant insights? Because there is not significant effect, does not mean there is no relevant insight. The major finding of the study is that, similar to the calcification response, respiration rates remain constant over a large pCO2 gradient. We think that, at the present state of knowledge, this is a relevant and important finding as it actually contradicted our expectations (stated in the Introduction) that an increase in respiration likely occurred to account for the potentially higher energy requirements in pH upregulation in the calicoblast layer.

R1: "The tested hypothesis that the ability to maintain positive rates of calcification (Maier et al 2013) at high pCO2 is accompanied by higher energy requirements could not been fully proved by measuring only the respiration rates as here presented. Other measurements such as food ingestion rates, and the lipid reserves production and deployment should be investigated in order to support the main hypothesis."

-> Absolutely right, we show, that respiration remained constant with respect to pCO2 (not in the time course of experiment, where we find a concomitant increase in respiration in all pCO2 treatments). Certainly future studies need to address why this homogeneity in respiration rates was found over the large gradient of pCO2 by measuring other metabolically relevant variables. However, not everything can be done in a first assessment. We now know that, quite unexpectedly, respiration rates do not indicate higher energy requirements that we thought would be needed to maintain calcification constant even at relatively high pCO2 levels. So, certainly other potential mechanisms need to be explored, of which we propose / discuss several in the manuscript.

R1: "In addition, there are two major factors which might have masked the response of

corals to the acidified treatments: i) as clearly showed by the high deviation of data, the parameter used to normalise the respiration rates (skeleton weight) is not the best one, as also recognised during the discussion; I know that measuring the protein content required the sacrifice of the precious samples. However, normalising data to the protein content or tissue surface area would be more informative than the skeletal weight"

-> Normalisation is an important issue, but we used a repeated measures design, where respiration rates were assessed for the same coral fragments repeatedly, so normalisation is not the problem as the variation and high deviation from mean also occurs within one and the same coral and the same pCO2 treatment.

R1: "ii) the high feeding regime used during this experiment certainly contributed to keep the corals happy, leading to more energy available for their growth. Actually we ignore the amount and quality of food for cold water corals in their natural environment but it is likely that feeding in aquaria does not mimic a real, ecological relevant situation.

-> Unfortunately, it is very difficult to ascertain the real, ecologically relevant situation in the field and there is no evidence that our feeding regime in the laboratory would be extremely high (or low) compared to the field. The effect of feeding was not addressed in this study. What is important with respect to feeding for the present study is that corals in the four different pCO2 treatments received similar amounts of food – which led possibly to the concomitant increase in respiration and calcification during the first couple of months for corals within all of the pCO2 treatments. However, as long as we do not have much indication with respect to the quantity and frequency of food being supplied to cold-water corals in their natural environment, we decided to utilize quantities that seem to make them "happy" under aquarium conditions. Naturally, we cannot mimic in the laboratory many of the other parameters that these deepwater corals might experience in their natural environment and that might be important for physiological performance (changes in temperature, currents, salinity, sedimentation, pressure, etc).

R1: "...This crucial aspect has not been extensively discussed so far, especially in the conclusions raised by the Authors, except in the last sentence of the section 3.3 which, in my opinion, is very dangerous because it invalids the conclusions and the message given in present study and the previous Maier et al 2013."

-> We do discuss the aspect of feeding – but address it "carefully". We did not study the effect of feeding (or starvation) in this study, so we cannot extensively discuss this aspect of the work. We used one feeding regime with prey equally distributed to corals within the 4 pCO2 treatments. We can therefore clearly exclude availability of prey as a factor that differed between treatments and that could have masked the respiration response at different pCO2 levels. There were no differences in food (energy) supplied at different pCO2 levels.

R1: "Minor comments Introduction. The saturation state of calcium carbonate is no more a good indicator of whether calcifying organisms can build and maintain their exoskeletons, as suggested by the Authors. There are several examples of corals able to calcify and growth without any signs of dissolution at undersaturated conditions (e.g. for DSC, Thresher et al 2011; Jantzen et al 2013 Mar Biol; Lunden et al 2013 L & O)"

-> The saturation state of aragonite will always remain a crucial parameter to (cold-water) coral calcification as it is the chemical base for carbonate precipitation and dissolution. What the reviewer refers to is the ASH (aragonite saturation horizon or the aragonite saturation state of 1), for which there is now growing evidence, that CWC are able to calcify below the ASH (or omega aragonite <1). This is addressed in the introduction (p. 7620, line 3ff). While the papers by Thresher and Jantzen, show occurrences of solitary CWC at $\Omega a < 1$. There is as yet no in situ evidence for the branching M. oculata or L. pertusa occurring at $\Omega a < 1$ (however, experimental evidence - as cited in the introduction and discussion section). The paper by Lunden reports Lophelia in situ occurrence at $\Omega a > 1$ (range: 1.25-1.60 for L. pertusa from the Gulf of Mexico).

R1: "I found the incubations in the respiration chambers too long. Leaving corals for

two hours and half in a closed vial means that most of the oxygen was consumed by the animals, likely becoming a toxic environment, and that the seawater carbonate chemistry of the medium greatly changed during the incubation, including the tested level of pCO2, therefore confounding the effect of treatments. Why the Authors used such long-term incubation?"

-> The oxygen saturation at the end of measurements was always above 88% and well above 90% for most of the incubations. We will add this info to the revised version of our manuscript. Knowing the volume of the chambers, one could calculate the decrease from 100% that occurred but it is much easier to supply this missing information – otherwise it might be confusing for readers. We should state that 2.5 hrs is not necessarily a long incubation time (particularly at the temperatures and chamber volume utilized). There were no issues with hypoxia.

For carbonate chemistry: We provide a thorough overview of the changes in carbonate chemistry taking place during the closed system incubation (Section 3.1. and table 3) which show that pCO2 increases by ca. 6% and omega Ωa decreases by ca. 5% and changes of all other parameters of the carbonate chemistry are below 1%, which means the carbonate chemistry between treatment levels remained clearly distinct from each other and is not, as the reviewer states, confounding the effect of treatments.

R1: "In section 2.3 there is confusion with the previous publication, including G, calcification rates, in the calculation. It is not clear from this section how the Authors measured it during the present experiment."

-> We will clarify in methods section: the method used for calcification is that described in PLOS One (Maier et al., 2013), and, for the present manuscript, only the calcification rates (and parameters of carbonate chemistry) measured in parallel with the respiration rates were taken into consideration when correlating G and R.

R1: "Regarding the hypothesis that more energy must be allocated to up-regulate

internal pH, McCulloch et al 2012 calculated for cold water corals a growth-rate cost of ca. 10 % per 0.1 pH unit decreases in seawater pH. This means a 20-40% increase in the present experiment, which likely was masked by the elevated feeding regime used and/or the large deviations of data."

-> We do not know, if the feeding provided to the corals would correspond to an "elevated feeding regime" compared to in situ. Nevertheless, even if it was elevated, it was similar among all the treatments. Using our repeated measures design, we should have been able to detect an effect of pCO2 on respiration rate. We observed no offset / increase in respiration associated with high pCO2 levels.

R1: "The Authors did not really discuss their results with previous studies on other Mediterranean cold water corals such as Naumann et al 2013, which showed high thermal tolerance of these corals."

-> The present work does not examine temperature effects and thermal tolerance limits. We only marginally addresses and discusses this aspect by including some supplementary data. Note, we take into account the publication on thermal tolerance by Naumann et al. (2013).

Interactive comment on Biogeosciences Discuss., 10, 7617, 2013.