

## ***Interactive comment on “Ocean acidification increases photosynthate translocation in a coral–dinoflagellates symbiosis” by P. Tremblay et al.***

**P. Tremblay et al.**

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Interactive comment on “Ocean acidification increases photosynthate translocation in a coral–dinoflagellates symbiosis” by P. Tremblay et al.

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This is not a full review, just an open comment providing some suggestions on this interesting manuscript.

Comment: The title and first paragraph of the introduction set the experiments in the  
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context of anthropogenic ocean acidification and the abstract concludes “this decrease might have important consequences for the survival of corals under an acidification stress”. Yet, the perturbation used, pH of 7.2 and pCO<sub>2</sub> of almost 4000  $\mu$ atm, are not relevant in this context. Barry et al. (2010) provide guidelines on the experimental design of ocean acidification perturbation experiments. I suggest that the title, abstract and introduction should be revised in order to avoid misleading the readership.

Response: In agreement with JP Gattuso’s comments: - We have changed the title from “Ocean acidification increases photosynthate translocation in a coral–dinoflagellate symbiosis” into “Photosynthate translocation increases in response to low seawater pH in a coral–dinoflagellate symbiosis”.

- We have changed the abstract as follow: we have replaced the sentence “Symbiont photosynthesis contributes for a large part of the carbon acquisition in tropical coral species and is therefore an important process that may determine their survival under climate change scenarios” by the sentence “Symbiont photosynthesis contributes for a large part of the carbon acquisition in tropical coral species. It is thus important to know how environmental changes affect this carbon acquisition and allocation in corals”. We have also replaced the sentence “In the long-term, this decrease might have important consequences for the survival of corals under an acidification stress” by the sentence “In the long-term, such decrease in symbiont concentration might severely affect the carbon budget of the symbiotic association”.

- In the introduction, we have replaced the sentence “symbiont photosynthesis is important for carbon acquisition in corals and is therefore an important process that will determine the survival and fitness of corals under the scenario of climate change” by the sentence “However, symbionts, through photosynthesis, are the major actors in nutrient acquisition of the symbiotic association (Muscatine et al., 1981, 1984; Grover et al., 2002), which in turn is a key process in corals and in animal in general that determines their growth, fecundity and survival. We have also added reference of previous experiments performed at very low pHs and added the following sentence to explain

the use of a low pH: "Although the low pH value chosen in this study is lower than that expected under future climate scenarios, it is useful to examine the physiological response of corals to environmental hypercapnia (Barry et al., 2010). It also helps constrain their boundaries of performance (e.g. Kurihara & Shirayama, 2004) to guide subsequent studies on similar taxa".

- In the Material and Methods, we also specified that: "From previous studies (Krief et al., 2010), we know that *S. pistillata* grows well under both pHs for years and the difference between 7.2 and 8.1 allows us to clearly established if there is a pH effect on the autotrophic carbon budget of *S. pistillata*".

Comment: It is absolutely essential to mention the pH scale used (pHNBS, pHT, pHf or pH<sub>SWS</sub>) every time a pH value is reported (see Pesant et al., 2010). Yet, the pH scale is not mentioned at all in this manuscript. The reader is referred to the discussion paper of Cohen and Fine (2012) which used the NBS scale and converted the values on the total scale using a very approximate approach.

Response: We agree with Jean-Pierre Gattuso and now, the pH is expressed according to two scales (NBS and total scale). 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). However, 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) is very complex. Given these difficulties, the pH<sub>NBS</sub> were shifted onto the total pH scale (pHT) 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).

Comment: Arbitrary values are used for the photosynthetic and respiratory quotients (respectively 1.1 and 0.8). There are very few values reported in the literature for

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scleractinian corals but data are available for the species investigated here (*Stylophora pistillata*) from a very close location in the Gulf of Aqaba (Gattuso and Jaubert, 1990). These values range from 1 to 1.5 for PQ<sub>net</sub> and from 0.7 to 1 for RQ<sub>c</sub>. I am not sure whether using these values would significantly alter the results and conclusions but the authors may want to address this concern. I hope that the authors will find these comments useful.

Response: The values used in the paper (PQ of 1.1 and RQ of 0.8, from Muscatine et al. 1981) are within the range of values found by Gattuso and Jaubert (1990) for *Stylophora pistillata* from the Red Sea, incubated at different depths. They also closely match values specifically obtained by Gattuso and Jaubert (1990) for colonies of *S. pistillata* collected at 5 m depth (PQ of 1.05 and RQ of 0.8). These colonies experienced ca. the same irradiance (162  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) as in this study (140  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ). Moreover, it has also been evidenced recently that glucose is the major transferred metabolite in dinoflagellate-cnidarian symbioses (Burriesci et al., 2012), and carbohydrates typically result in a PQ of 1.16, very close to the value used in this work. Most of these photosynthates are respired by the colony of *S. pistillata* (Tremblay et al., 2012b), and the RQ associated to the respiration of carbohydrates is 0.86, also very close to the value used in this study.

We however agree that Gattuso and Jaubert (1990) is a better reference than Muscatine et al. (1981) for this coral species and we have added it to the manuscript. Concerning the changes in the carbon budget induced by the PQ and RQ values considered: Depending on the values considered, there might be a slight over or under-estimation of the carbon fixed or respired (called PC and RC in the model of Tremblay et al. 2012). In any case, the amounts of carbon incorporated into the symbionts and the host are not affected, as they are directly measured using the tracer <sup>13</sup>C. The other parameters might change as follow (for each pH condition):

If PC and RC are both underestimated and true values are higher: The amount of carbon fixed in the symbiont or the host (CR) will slightly decrease, The amount of carbon

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lost (CL) as respiration and mucus (POC and DOC) release will slightly increase, The amount of carbon translocated (TS) will remain unchanged because it is the difference between PC and RC.

If PC and RC are both overestimated and true values are lower: The amount of carbon fixed in the symbiont or the host (CR) will slightly increase, The amount of carbon lost (CL) as respiration and mucus release will slightly decrease, The amount of carbon translocated (TS) will remain unchanged.

If PC is lower than estimated and RC is higher: The amount of carbon fixed in the symbiont or the host (CR) will slightly increase, The amount of carbon lost (CL) as respiration and mucus release will slightly decrease, The amount of carbon translocated (TS) will slightly decrease.

If PC is higher than estimated and RC is lower than estimated: The amount of carbon fixed in the symbiont or the host (CR) will slightly decrease, The amount of carbon lost (CL) as respiration and mucus release will slightly increase, The amount of carbon translocated (TS) will increase.

Overall, differences between pH conditions will remain unchanged, and corals incubated under low pH will still have a higher photosynthate translocation compared to corals maintained under normal pH, because: - PC and RC in each condition will change in the same direction (if they are over or under-estimated, they will be in each pH condition), - The difference in photosynthetic rates between the two pH conditions is large (25% higher under normal than low pH), leading to the difference in photosynthate translocation.

The carbon budget will also remain unchanged as: - The incorporation rates in the symbionts and coral host do not depend on the PQ and RQ coefficients. Thus, incorporation rates at pH 7.2 will remain twice lower than at pH 8.2, - Translocation rates will remain the same as calculated, i.e. a higher translocation rate for corals incubated at pH = 7.2, - There might be only a slight change between the amount of carbon retained

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in the coral tissue and the amount of carbon lost. However, since the amount of carbon lost is huge (60 to 73%) compared to the carbon remaining in the tissue, the results and conclusions will not be significantly altered.

We hope that we have answered positively to JP Gattuso comments.

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Interactive comment on "Ocean acidification increases photosynthate translocation in a coral–dinoflagellates symbiosis" by P. Tremblay et al.

Anonymous Referee #1

Received and published: 31 January 2013

General Comments

Tremblay et al., present an interesting examination of carbon production, translocation, and loss in corals and Symbiodinium under different seawater pH conditions. This study would be strengthened by inclusion and statistical analysis of all time points and response variables for a more complete picture of carbon budgets. In addition, a pH of 7.2 and a  $p\text{CO}_2$  of  $4000 \mu\text{atm}$  are extreme far future scenarios for OA and should be discussed as such.

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Comment: There are no methods or background provided for any of the seawater chemistry analysis. How often were pH and alkalinity sampled through the 6 months and during the incubations? How were they sampled and processed/analyzed?

Response: We agree with the reviewer and have added the following paragraph in the Material and Methods section: "...seawater was continuously pumped at 30 m depth into several 1000 l tanks, each equipped with a pH electrode (S-200C, Sensorex, CA, USA) connected to a pH controller (Aquastar, IKS Computer System GmbH, Karlsbad, Germany). The controller continuously monitored pH and adjusted it to the desired value by bubbling  $\text{CO}_2$  (from a  $\text{CO}_2$  cylinder). Daily pH variability was low ( $\pm 0.05$ ) throughout the experiment and no major fluctuations were recorded. Well-mixed filtered ( $500 \mu\text{m}$ ) water from each tank continuously flowed into smaller tanks containing the coral nubbins. All pH data were recorded using monitoring software (Timo, Matuta, Germany) on the NBS scale. The pH<sub>NBS</sub> data were shifted onto the total pH<sub>T</sub> scale by subtracting 0.1, which includes a minor correction for  $[\text{SO}_4^{2-}]$  and the stability constant of  $\text{HSO}_4^-$  at a salinity of 40.7 (Krief et al., 2010).

During the incubation, we specified that: "The pH<sub>NBS</sub> was controlled after addition of the  $^{13}\text{C}$ -bicarbonate and during the whole experiment to ensure that it remained at the desired level, especially for the low pH condition".

Comment: It seems arbitrary to only statistically analyze initial and final time points, especially when it appears for some variables that the initial and final points don't differ as much as the middle time points (e.g., Figures 3 and 4). Is there a reason all time points are presented but not analyzed? Is there a difference in integrated carbon loss that is being obscured by only analyzing the initial and final time points?

Response: We agree with the reviewer and the statistical treatments have been changed accordingly. We have now tested the effect of pH and time points using a repeated measure analysis of variance, and the conclusions of the analysis, are not fundamentally different. We therefore changed the Methods section as follow:" The ef-

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fect of pH on the incorporation rates, on the percentage of fixed carbon remaining (CR), the carbon lost (CL) and the carbon translocation (TS) was tested using a repeated measure analysis of variance (ANOVA). Colonies were considered as “subjects” while pH was the between subject factor. The second factor was time over the course of the chase interval, and had five levels (0, 2, 4, 24 and 48 hours). The repeated measures ANOVA was followed by a posteriori test (Tukey’s test) if significant.” The results section has been changed accordingly.

In summary:

Incorporation rates and CR of the symbionts decreased with time (Fig. 3a,c and Table 3). Incorporation rates and CR of the coral host did not increase with time (Fig. 3b,d and Table 3). CL and TS increased with time (Fig. 4 and Table 4).

Comment: What symbionts does *S. pistillata* host? How specific or flexible is this, and would you expect it to change with CO<sub>2</sub> exposure after 6 months? Are your results due to symbiont type and not pH?

Response: Yes, we have indeed determined the symbiont clades in the colonies of *S. pistillata* used in this study. Symbiont phylogeny was determined to the clade level according to Santos et al. (2002), using chloroplastic ribosomal deoxyribonucleic acid sequences. Clade A was the dominant clade, both when colonies were sampled in the Gulf of Eilat at the beginning of the experiment and after six months at the two pHs. The best hit on NCBI was *Symbiodinium* sp. A (23S ribosomal RNA gene of chloroplast) isolated from a Caribbean gorgonian (*Plexaura kuna*; Accession number #JN558027; Pochon et al. 2012). The results are actually due to the pH and not to the symbionts clade.

We however agree that this is an important point and we have added the information to the Material and Methods section: “...the dominant clade of *Symbiodinium* hosted by colonies of *S. pistillata* used in this study was determined to the clade level according to Santos et al. (2002), using chloroplastic ribosomal deoxyribonucleic acid sequences.

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In colonies sampled in situ, as well as in nubbins maintained at the two pHs, clade A was dominant and did not change during the incubation.

Comment: Not all statistical values appear to be available in text or table. Table 3 could be expanded from only two variable and two time points to include all time points for the four variables of incorporation, carbon remaining, carbon lost, and translocation.

Response: We have now tested the effect of pH and time points on the four variables of incorporation, carbon remaining, carbon lost, and translocation using a repeated measures analysis of variance (see comment above). Table 3 have been expanded and Table 4 have been added, and all statistical values or results appear in the text, in Tables 3 and 4, and Fig. 3 and 4 (posteriori test).

Technical Corrections:

Page 85, Line 16: Replace “for” with “to” We have changed the sentence.

Page 85, Line 18: Not the only taxon with form II Rubisco (Zhang and Lin, 2003; Robinson et al., 2003; Tourova et al., 2010) We have changed the text accordingly.

References

Krief, S., Hendy, E. J., Fine, M., Yam, R., Meibom, A., Foster, G. L., and Shemesh, A.: Physiological and isotopic responses of scleractinian corals to ocean acidification, *Geochimica Cosmochim. Acta*, 74, 4988-5001, doi:10.1016/j.gca.2010.05.023, 2010. Pochon, X., Putnam, H. M., Burki, F., and Gates, R. D.: Identifying and characterizing alternative molecular markers for the symbiotic and free-living dinoflagellate genus *Symbiodinium*, *PLoS ONE*, 7, e29816, 2012. Robinson, J. J., Scott, K. M., Swanson, S. T., O’Leary, M. H., Horken, K., Tabita, F. R., and Cavanaugh, C. M.: Kinetic isotope effect and characterization of form II RubisCO from the chemoautotrophic endosymbionts of the hydrothermal vent tubeworm *Riftia pachyptila*. *Limnology and oceanography*, 48-54, 2003. Santos, S. R., J. , T. D., Kinzie, R. A., Hidaka, M., Sakai, K., and Coffroth, M. A.: Molecular phylogeny of symbiotic dinoflagellates in-

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Interactive comment on "Ocean acidification increases photosynthate translocation in a coral–dinoflagellates symbiosis" by P. Tremblay et al.

Anonymous Referee #2

Received and published: 8 March 2013

Comment: This paper is an intriguing and timely addition to the field of coral physiology. The paper is well written and does a good job leading the reader through relatively complex methods and results. The statistical design is appropriate for the data and ample relevant literature was used to provide this study context. My only major suggestion is to reevaluate the characterization of this study as an "ocean acidification" study both in the title, and throughout the paper. I elaborate further in comment #2 below. Despite that, I feel the work is strong and I recommend only minor changes before publication.

Response: Jean-Pierre Gattuso has already suggested to revise this point and you will find below the list of changes made in the paper: - We have changed the title from "Ocean acidification increases photosynthate translocation in a coral-dinoflagellate symbiosis" into "Photosynthate translocation increases in response to low seawater pH in a coral-dinoflagellate symbiosis".

- We have changed the abstract as follow: we have replaced the sentence "Symbiont photosynthesis contributes for a large part of the carbon acquisition in tropical coral species and is therefore an important process that may determine their survival under

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climate change scenarios" by the sentence "Symbiont photosynthesis contributes for a large part of the carbon acquisition in tropical coral species. It is thus important to know how environmental changes affect this carbon acquisition and allocation in corals". We have also replaced the sentence "In the long-term, this decrease might have important consequences for the survival of corals under an acidification stress" by the sentence "In the long-term, such decrease in symbiont concentration might severely affect the carbon budget of the symbiotic association".

- In the introduction, we have replaced the sentence "symbiont photosynthesis is important for carbon acquisition in corals and is therefore an important process that will determine the survival and fitness of corals under the scenario of climate change" by the sentence "However, symbionts, through photosynthesis, are the major actors in nutrient acquisition of the symbiotic association (Muscatine et al., 1981, 1984; Grover et al., 2002), which in turn is a key process in corals and in animal in general that determines their growth, fecundity and survival. We have also added reference of previous experiments performed at very low pHs and added the following sentence to explain the use of a low pH: "Although the low pH value chosen in this study is lower than that expected under future climate scenarios, it is useful to examine the physiological response of corals to environmental hypercapnia (Barry et al., 2010). It also helps constrain their boundaries of performance (e.g. Kurihara and Shirayama, 2004) to guide subsequent studies on similar taxa".

- In the Material and Methods, we also specified that: "From previous studies (Krief et al., 2010), we know that *S. pistillata* grows well under both pHs for years and the difference between 7.2 and 8.1 allows us to clearly established if there is a pH effect on the autotrophic carbon budget of *S. pistillata*".

Comment: Page 5 line 7 – Please include the pH units in the methods in addition to what you have already included in Table 1.

Response: The pH is now expressed according to two scales (NBS and total scale).

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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). However, preparation of synthetic seawater for total scale measurements with the high ionic strength of the Red Sea ( $\sim 0.85$  compare to  $\sim 0.7$  of oceans; Zeebe and Wolf-Gladrow, 2001) is very complex. Given these difficulties, the pH<sub>NBS</sub> were shifted onto the total pH scale (pHT) 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).

Comment: Also please include with what regularity seawater chemistry was sampled.

Response: We agree with the reviewer and have added the following paragraph in the Material and Methods section: "Seawater was continuously pumped at 30 m depth into several 1000 l tanks, each equipped with a pH electrode (S-200C, Sensorex, CA, USA) connected to a pH controller (Aquastar, IKS Computer System GmbH, Karlsbad, Germany). The controller continuously monitored pH and adjusted it to the desired value by bubbling CO<sub>2</sub> (from a CO<sub>2</sub> cylinder). Daily pH variability was low ( $\pm 0.05$ ) throughout the experiment and no major fluctuations were recorded. Well-mixed filtered (500  $\mu\text{m}$ ) water from each tank continuously flowed into smaller tanks containing the coral nubbins. All pH data were recorded using monitoring software (Timo, Matuta, Germany) on the NBS scale. The pH<sub>NBS</sub> data were shifted onto the total pHT scale by subtracting 0.1, which includes a minor correction for [SO<sub>4</sub><sup>2-</sup>] and the stability constant of HSO<sub>4</sub> at a salinity of 40.7 (Krief et al., 2010).

During the incubation, we specified that: "The pH was controlled after addition of the <sup>13</sup>C-bicarbonate and during the whole experiment to ensure that it remained at the desired level, especially for the low pH condition".

Comment: Page 5 line 10 - 140  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  were used in the experimental

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conditions. Were any in situ light levels recorded from where the three colonies of *S. pistillata* were collected prior to the start of the experiment? If so, please include them as well.

Response: We have added this information in the Material and Methods section: "The in situ light level was approximately equal to 280  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  at noon."

Comment: Page 11 line 7 – The "important seawater acidification (pH 7.2). . ." is arguably not an important seawater acidification level. This work, although novel and profound in its own right, cannot be directly compared to the vast majority of other OA studies, which commonly utilize pCO<sub>2</sub> levels of 800-1200  $\mu\text{atm}$  as their low pH/high CO<sub>2</sub> treatment. This does not mean studying the response of the coral holobiont physiology to extremely high pCO<sub>2</sub> conditions is not useful as long as it is addressed in a transparent manner, highlighting the limitations of the experimental conditions chosen. Indeed the fact that the coral host ultimately acquired the same amount of autotrophic carbon under both pH's was especially surprising given the extremely low pH treatment used. Discussing the limitations of the experimental conditions used should be added.

Response: We agree with the reviewer comment. It's why we have added the following sentences: - In the introduction: "Although the low pH value chosen in this study is lower than that expected under future climate scenarios, it is useful to examine the physiological response of corals to environmental hypercapnia (Barry et al., 2010). It also helps constrain their boundaries of performance (e.g. Kurihara and Shirayama, 2004) to guide subsequent studies on similar taxa". - In the material and methods section: "From previous studies (Krief et al., 2010), we know that *S. pistillata* grows well under both pHs for years and the difference between 7.2 and 8.1 allows us to clearly establish if there is a pH effect on the autotrophic carbon budget of *S. pistillata*". - We have also changed some sentences in the Discussion, such as the one highlighted by the reviewer on p 11 line 7 and we have also changed the conclusion saying: "Questions remaining open are whether the symbiotic association can remain on the fragile equilibrium observed in this study on the long-term or if the symbiont physiology will

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further decline, or how the symbiotic association is impacted when maintained on a very long term under a smaller pH/pCO<sub>2</sub> perturbation, and what will be the cross effect of increased pCO<sub>2</sub> and seawater temperature on the carbon translocation and budget in such symbioses.”

Comment: What was the overall health/condition of the coral and skeleton during this experiment? A qualitative description of general health and skeletal condition after a long-term exposure to such low pH levels would aid the reader in contextualizing your results.

Response: The following sentence has been added to the Material and Methods section: “From previous studies (Krief et al., 2010), we know that *S. pistillata* grows well under both pHs for years and the difference between 7.2 and 8.1 allows us to clearly established if there is a pH effect on the autotrophic carbon budget of *S. pistillata*”.

Comment: I particularly appreciate Fig. 5. I feel it greatly adds to understanding the rather complex interactions between host and symbiont between the two treatments.

Response: Thank you.

#### References

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