Dear editor, dear reviewers,

We thank you for the time spent on our manuscript. We have now prepared a new version of our manuscript (BG-2019-356) according to the comments made by the reviewers. Changes made to the text are detailed below.

Answer to Report #1

Submitted on 30 Jun 2020
Referee #3: Takashi Toyofuku

Reviewers' comments are displayed in regular style whereas the author's reply is in italic bold.

General comments:

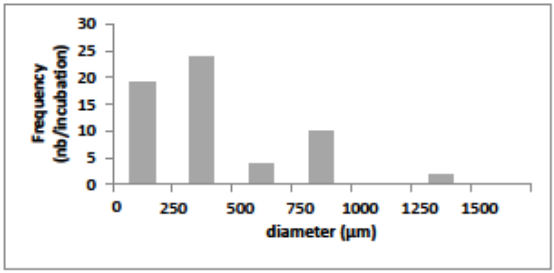
I like this study very much.
This study is highly commendable because it an elaborate experimental design with reliable experimental techniques by well-established laboratory. The experimental results are robust. The results are novel and are of interest to many audiences

We thank the reviewer for his kind words and constructive assessment. Below, we indicated how we changed our manuscript based on the comments

Specific comments:

L78-79: Could the authors show the size distribution of each experimental condition in the supplemental materials?

We added a figure to the supplementary material to show the size distribution per group. Since we do not know whether juvenile/ adult specimens respond differently to the treatments, we decided to incubate specimens large size range. This way we avoid a potential bias when extrapolating results to specimens from a specific size range. We now highlight this in the new version of our manuscript: line 81. : "After a week, viable specimens were collected and divided over eight experimental conditions, each of them consisting of three groups (Fig. 1). Each group consisted of 40-60 specimens with a similar size distribution (initial diameter: 140 to 1200 µm, shown in S1). "



S2: Size distribution of the individuals at the beginning of the experiment

L94 Authors should indicate why they have quantified the amount of precipitation from DIC and alkalinity. It is clearly worth stating that there is no other way to estimate it precisely.

We thank the reviewer for this suggestion and have added this precision at L96-98 : "This method was chosen above other growth method measurement such as sample weighing (which is destructive) or chamber count as it allows a quantification of the amount of calcite formed during the experiment."

L114 Authors should explain the meaning of the color of the arrows and the dotted lines in the captions. The same manner in later figures 3 and 4.

We followed the reviewer's suggestion and added precisions to the description of the figure. See new graph and caption added below.

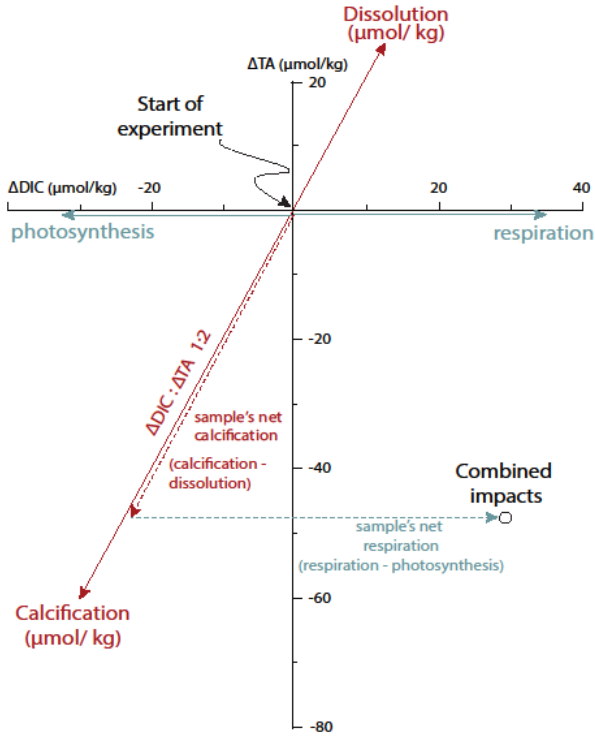


Figure 2: Calcification and net respiration of foraminifera deduced from changes in DIC and total alkalinity over time. The blue vectors show the impact of photosynthesis and respiration (impacting DIC), the red arrow show the impact of calcification and calcite dissolution (impacting both DIC and TA in a 1:2 ratio). Observed changes for each incubation should be decomposed into two vectors: a contribution of calcification (dashed red arrow) and the net effect of

respiration and photosynthesis (dashed blue arrow). Approach is indicated here for a hypothetical incubation

I would like to see the photos of individuals grown in the control, AZ, and DCMU conditions.
SEM pictures of individuals grown under different conditions have been added to the supplementary information (figure S1).

L184 As a previous reviewer pointed out, calcification is thought to require energy. It's hard to distinguish whether the problem is a shortage of energy or the insufficiency of photosynthesis itself.

Given the dark conditions every 12 hours, I'm not sure that the competition for carbon between photosynthesis and calcification is a problem. It should also be pointed out that sharing the CO2 by time may be occurring.
We agree with the reviewer's comment and added this precision L202-204: "As the foraminifera were in the dark 12h hours a day it is feasible that DIC is shared over time, being used for calcification during the dark phase and for photosynthesis during the light phase. "

For example, would calcification have been enhanced even if there were no dark conditions for 24-hour? Authors don't need to answer this question, but I think this sort of question would be helpful to sort out the really dominant factors
In the pre-experimental period when frozen algae were

given, how much numbers of chambers were added?

We have not quantified number of chambers added in the time before starting the experiment. We assume that the number of chambers added during the pre-experimental period is similar to the number of chambers added during the experiment under "control" conditions (one or two chambers).

L220 CA is an enzyme that is extremely universally found in the cytoplasm. I do not deny that CA is involved in calcification process, and I also believe so. However, the possibility that the activities of CAs of non-calcification site may also affect calcification. The possibility of widespread inhibition of the metabolic activity should be clearly described.

We agree with the reviewer and have stressed this in the Discussion of our manuscript L236-237: ". It is also likely that cytoplasmic CAs -involved for instance in intracellular pH regulation- also affect calcification."

Report #2

Referee #4: Anonymous referee

General comments:

A paper by de Goeyse et al. conducted a simple incubation experiment to test the role of carbonic anhydrase (CA) using the inhibitor acetazolamide on the calcification of symbiont-bearing foraminifer Amphistegina. Although the results clearly show the

involvement of CA on calcification of the foraminifer, it is still a vague impression to me where CA is present at the surface of cell membrane or the site of calcification. I look forward to authors' future cellular-scale studies to solve this question.

Some technical corrections are necessary prior to the acceptance of this paper.

We thank the reviewer for the assessment of our manuscript: below, we answer point-by-point to the comments.

Specific comments:

L11: Symbiont>symbiont
L17: seawater is taken up > how?
L38: Hikami et al., 2011)) > delete the last)
L51: SOC > site of calcification (SOC). Spell in full when first mentioned in the text
L54: the conversion from HCO3- > the conversion from HCO3- outside the test/cell membraned?

We have changed the text of our corrected manuscript accordingly and thank the reviewer for pointing out these elements.

L55: This process may be catalyzed by an enzymatic conversion by carbonic anhydrase (CA) > Does this process occur at SOC?
This is indeed an important issue and unfortunately, we cannot solve this with the current dataset. As AZ is not

membrane-permeable we here hypothesize that the enzyme is located within and/or at the outer cell membrane.

L79: similar size distribution (initial diameter: 140 to 1200 µm) > too broad initial diameter
We did not want to suggest that the sizes of all incubated specimens were similar, but rather that the sizes (and size distribution) were similar between groups. We have changed the wording here and, also in reply to the first reviewer, added an additional figure showing the sizes of the incubated individuals.

L103: (Liu et al., 2015) > Liu et al. (2015)
L119: the first one is constant the second present > the first one is constant and the second one is present

L144: many specimens in the control vials added 2 or 3 chambers > According to Table 3, most specimens added 1 or 2 chambers; only one specimen added 3 chambers.

L152: 42 and 16 > 16 and 42

L153: only 22 (resp. 19) µmol·L−1 > only 19 and 22 µmol·L−1, respectively
L163: approximately 65 µmol·L−1 > Where does this figure comes from ?

L165: 60 µg·Ind.−1·day−1; > Delete ;
L168: 0.3-6.6 > Add unit
L173: Fig. 2 > Fig. 3?
L196: (Ter Kuile et al., (1989b, 1989a) > Ter Kuil et al.

(1989a, 1989b), but no 1989a, b in References
L201-203, 210: Cited references are not listed in the References.
L205: in known as > delete in
L221: In for example > For example
Text and references have been corrected accordingly

L256: not light, but photosynthesis itself promotes calcification in perforate foraminifera. > better to say "… in symbiont-bearing perforate foraminifera". I suggest that you should pay more attentions to differences between light and dark respirations to understand light-enhanced calcification.
We have changed the sentence L256 according to the reviewer's suggestion

Fig. 4 caption: Arrows show the calcification (red) and net respiration (blue) effects. > Add this sentence in Fig. 2 and 3 as well.

Figure and figure caption changed accordingly. New caption now reads: " Calcification and net respiration of foraminifera deduced from changes in DIC and total alkalinity over time. The blue vectors show the impact of photosynthesis and respiration (impacting DIC), the red arrow show the impact of calcification and calcite dissolution (impacting both DIC and TA in a 1:2 ratio). Observed changes for each incubation should be decomposed into two vectors: a contribution of

calcification (dashed red arrow) and the net effect of respiration and photosynthesis (dashed blue arrow). Approach is indicated here for a hypothetical incubation"

Table 3: I am wondering if these specimens are dead or alive after incubation.

Because of the limited during of the experiments (<5 days) (almost) all foraminifera were alive after the incubation. This has not been quantified, but no differences were observed between experiments. A preliminary experiment was performed prior to this study to make sure that the concentration of the inhibitor used did not affect foraminiferal survival. That experiment showed all foraminifera alive over a time span of a week.