

We thank the reviewer for acknowledging our manuscript as important, interesting, and adequate for publication. We agree with the comments and suggestions by this reviewer and we believe we were able to positively respond to all mentioned issues, and to revise the manuscript accordingly. Below is a point-by-point reply (in blue).

General evaluation:

This elegant study presents data of a laboratory experiment comparing, for 2 larger symbiont-bearing benthic foraminiferal species, their response to high temperature, in terms of the foraminiferal calcification rate and the photosynthetic rate of their symbionts.

These data are important, interesting, and deserve to be published. The study is very well conceived, and the high quality data are analysed with adequate statistical methods.

The text is rather short, and the information is quite dense. In such a case, the written text should be very precise, and all potential sources of ambiguity should be avoided.

This is not always the case yet.

A main, recurrent problem in the discussion is that systematically, there is confusion between the holobiont (foraminiferal host + symbionts) and the host (the foraminifer without symbionts). Often, the authors speak about the host, when they mean the “whole foraminifer”, that is the holobiont. This is not surprising, because it is probably impossible to consider the foraminifer without its symbionts, which represent an essential part of it. For that reason, I think it's impossible to compare the “well-being” of the foraminiferal host with that of the symbionts!

This becomes problematic when the calcification rate alone is supposed to represent perfectly well the general state of the “foraminiferal host”. Most times, when the authors compare “the host and the symbionts”, in reality, they compare the “calcification rate of the foraminifer” with the (photosynthesis rate of the) symbionts.

I agree that the photosynthetic rate probably describes the health of the symbionts very well, but I am not convinced that the same can be said about the foraminifer and its calcification rate. I would say that many other factors (together) determine (and can inform about) the wellbeing of the foraminiferal holobiont, the wellbeing of its symbionts being one of them!

Summarising, the authors should formulate things more carefully. They compare foraminiferal calcification rates with symbiotic photosynthetic rates. Then that's what they should write!

Response: We agree with this point and adjusted the text accordingly. See specifics in the detailed comments below.

Similarly, I think that some parts of the discussion go too far. The authors have only tested part of the response of the foraminiferal holobiont to high temperatures. Other indicators (locomotion, feeding, reproduction, etc.) may respond differently, and future climate change will probably lead to changes in other stressors (salinity, oxygenation, carbonate chemistry, etc.) as well. They should therefore be much less affirmative when they discuss the future evolution of larger BF communities.

Response: We agree with this point and changed it throughout the text. We specifically address the issues of other well-being indicators and of other stressors in the detailed comments below (comment 2 and 23).

Detailed comments:

1. Line 12: a “contribution to thermal tolerance” is somewhat strange. This suggest some pro rata contribution to the tolerance of several factors. In reality I would expect that the overall tolerance is determined by the element which is least tolerant, either the foraminifer or its symbionts.

Changed to: "In order to assess the holobiont thermal tolerance we separately evaluated foraminiferal calcification rates with symbiotic photosynthetic rates"

2. Line 13: a key question is to what point calcification (for foraminifera) and photosynthetic activity (for symbionts) can be considered representative for their tolerance. For the symbionts, since photosynthesis is a primary life process, this is probably the case. Concerning foraminiferal calcification, it is less evident that this is the best marker of tolerance. I would say overall activity (feeding, locomotion, pseudopod movements) and reproduction are more critical parameters. There are many observations of (active?!) foraminifera under stressed conditions without calcification (even of decalcified forams). Only on the long term, a lack of calcification may lead to disappearance. I think this point should be discussed.

Previous studies have demonstrated that calcification rates can be used as a direct parameter for comparing the temperature sensitivity of different calcifying organisms, this is due to the fact that calcification involves a profound consumption of energy. Therefore, calcification rates are directly linked to the range of optimal to suboptimal conditions of the organism (Lough & Barnes, 2000; Carricart-Ganivet et al., 2012). This was specifically demonstrated on different species of foraminifera (Schmidt et al., 2011, 2015, 2016a, 2016b; Vogel and Uthicke ,2012, Uthicke and Fabricius, 2012, Evans et al., 2015).

The Alkalinity anomaly method present an ideal experimental approach for detecting even subtle differences in performance under different treatments and while it is true that the overall activity and reproduction are critical parameters to indicate the well-being of foraminifera they will not identify as clearly and as quantitatively the small differences between treatments. We added a short explanation of this in the method section.

3. Line 15: “sensitivity to 35°C”: what does that mean, if resilience is up to 32°C? The way it is formulated, the authors suggest that there is no sensitivity until 32°C which is evidently wrong. They probably mean something like “progressive loss of life functions between 32°C and 35°C”.

Changes as suggested.

4. Line 16: “future warming will change. . .). The word “will” is definitely too affirmative. Replace by “may”.

Changes as suggested.

5. Line 17: “a synchronized response”: this suggest that there is some deliberate process behind it, like host and symbionts coordinating their activities. Since you don’t know this, it is better to use the more neutral tem “synchronous”.

Changes as suggested.

6. Line 20: “pre-exposure to modest temperatures”. This is too imprecise. Should be “moderately high temperatures”.

Changes as suggested.

Introduction

7. Lines 28-29: No, it is not this area, but the entire Mediterranean which can be considered as a miniature ocean.

Changes as suggested.

8. Line 41: “Some species live close to their thermal thresholds”: upper or lower? In fact, the further invasion of some LBF (*Amphistegina*) in the Western Med is hampered by the fact that they are limited by their LOWER temperature threshold (at present, it is too cold for them to go further west). I guess that you mean here that in the Eastern Med, at present, they live close to their UPPER threshold?! Please be more specific.

The text is corrected to indicate upper thresholds. It is true that species are limited by their lower temperature threshold, but it is still important to consider upper threshold as even small increase in temperature, predicted in the relatively near future will influence species that are close to their upper thresholds.

9. Lines 43-44: “the relative contribution (positive or negative) of the host and symbiont algae to cope with rising temperatures”. As indicated before, this is really strange. The way it is written here, the sentence doesn’t make sense. You probably mean “the relative contribution to the tolerance of rising temperature”. But also that concept is very strange. This suggests that somehow you can quantify that when a LBF can still function at let’s say at 30°C, what proportion of this resistance is due to the foram itself, and

what proportion is due to (activities of) the symbionts. I think this is not possible! You simply want to investigate the “relative tolerance of host and symbiont algae to higher temperature”. With the underlying idea that the element with the lowest tolerance (host or symbionts) will probably be determinant for the tolerance of the holobiont.

Indeed, we mean that the component with lowest tolerance will limit the tolerance of the holobiont. We changed the wording to make it clearer.

10. Lines 45-46: “LBF species with different holobiont systems”: incorrect formulation: the LBF species IS the holobiont system (combination of host and symbionts)! The 2 species represent 2 different holobiont systems.

Changed.

## Methods

11. Lines 67-68: “however, they were used to produce comparable data to that of related published papers (Schmidt et al., 2016b, 2016a, 2018; Titelboim et al., 2019).”

This sentence is very unclear. “they” should be more specific, like “these light conditions”. But then, also the following part of the sentence doesn’t make sense to me. Who used these conditions to produce comparable data? You? Or the cited authors? But you say the data are comparable to data of these authors. So it is probably your data you talk about?! Then you should write: “however, while using these light conditions, we were able to produce data comparable to those presented in related published papers.”

Changed.

12. Finally, if I understand you right, I don’t see why the fact that you have comparable data as other others with the same conditions shows that these conditions are ok?! Maybe both your and other studies have unreliable results because by using insufficient light, you may have added an additional stress factor?! This eventuality should be discussed!

Ziegler and Uthicke, 2011 specifically indicate that LBF acclimate very rapidly to different light levels in under 48 hours. This means that our 10 days acclimation is sufficient for them to adjust to the specific light level provided during the experiment. Furthermore, it is important to note that the light level is low in respect to the photosynthetic optimum and not other physiological functions of the holobiont. Based on our own experience of culturing *Amphistegina* in the lab for several years we can confirm that specimens look healthy (colorized, strong motility), and show substantial growth by producing new chambers in similar manner as field specimens.

## 2.2 Laboratory manipulative experiments

13. Line 77: “acclimated under constant conditions”: it is essential so say at what temperature!

This is all described in the Supplementary and a reference to the specific table was added to the text.

### 2.3. Results

14. Fig. 2b: no data for photosynthetic activity of *S. orbiculus* in winter. Why not? Explain in methods section!

We had a technical problem with the measurements and decided not to include them in this data. We do not feel that this takes away from the strength of our results.

However, the caption of Suppl. Table S.3.2. mentions 3 groups!

In Suppl. Table S.3.2. there are only 2 groups.

15. Lines 115-119. "in the winter population, calcification decreases already after one week and is inhibited after three weeks"

This looks like an over-interpretation to me: in view of the overlapping error bars, I don't think that the "week 1" values are statistically different for 25°C and 35°C! The supplementary table doesn't inform us about this.

Since the first week decrease is not statistically significant, we have changed the sentence. This does not impair the point of the different populations as the main difference between them is the magnitude of decrease and not the point in time that it had happened in.

16. Fig. 3: I'm intrigued by the last line: "Abnormal measurement is marked as extreme and is not calculated as part of the average and error." I would write "a single abnormal measurement, obtained after x weeks. . .". You have to add the info in which week this measurement was made!

Added to the caption.

17. The regular text also describes this anomalous measurement and says "...as it is clearly damaged from sample handling". I don't see how inadequate sample handling can lead to such a value! I would simply not explain this single anomalous value.

This part is deleted.

### Chapter 3.2. *Amphistegina*

18. Line 131 "Both calcification and photosynthesis responses remain synchronized throughout the experiment".

I don't think you can say that. "Remain synchronized" means that there is an intrinsic interaction mechanism which explains why the responses of these two parameters are synchronous. "synchronised" is a wrong word. You should write: "are synchronous throughout the experiment".

Changes as suggested.

19. Line 133: “calcification and photosynthesis were both inhibited”.

However, calcification values are still slightly positive, so calcification doesn't seem to be (entirely) inhibited!

Inhibited is replaced with "severely reduced"

20. Line 133 “and net photosynthesis was negative”

That doesn't add anything to “inhibited photosynthesis”. If you want to mention this, it should come BEFORE the conclusion of inhibited photosynthesis.

Since the first part of the sentence is now changed from "inhibited" to "severely reduced", it does add information about the result of this reduction.

#### Discussion

Lines 143-145: “Specifically, our results predict that with rising temperatures the relative contribution of *S. orbiculus* will increase since its calcification is not inhibited even at extreme temperatures, contrary to *A. lobifera*”.

I have three problems with this sentence:

21.(1) I would prefer when, before jumping to such a conclusion, you first briefly summarise the differences you found between the two species.

We moved the paragraph summarising the differences between the species (this also refers to comment 24).

22.(2) Next, as said before, to me, the situation doesn't seem so “black-white” as you suggest (inhibition – no inhibition): for both species the calcification rate goes down at 35°C. It is true that the values go down much more for *A. lobifera*, but it doesn't become zero. If you think that a value of 10 µMol carbonate per individual per week means “no calcification”, then you have to explain why!

Calcification rate values of 10 µMol carbonate is within the precision of the alkalinity measurements in this study. In fact, ±10 µMol is the maximum error of our results. However, we accept this comment since values might be slightly higher than zero and rephrased inhibition with severely reduced.

23.(3) I think this conclusion goes much farther than you can go with your present results. I could imagine that a species no longer calcifies in the warmest month, but still survives these months without any major problems. Your observations only suggest that *A. lobifera* resists less well than *S. orbicularis* to high temperature. But that's not enough to go as far as you go, by concluding that in future, *Sorites* will progressively replace *Amphistegina*. In fact, temperature is one stress factor, but there may be others, which could covary with temperature, like salinity. Maybe the tolerance of the two species to raised salinity (or any other stress factor) is exactly the opposite?

We have adjusted the conclusions to better represent the scope and the significance of this study. Specifically, concerning the possible effect of salinity, our previous studies indicate that temperature is a much more prominent stressor than salinity (Titelboim et al., 2016, Kenigsberg et al., 2020). This is further supported by culturing experiment that is presently being carried out in our laboratory which is testing the separate and combined response of *A. lobifera* to elevated temperature and salinity. However, since this point is also true for other stressors and changes caused by future climate change, we have rephrased the conclusion.

24. Lines 159-166: there the authors summarise their main results. But this is way too late. This paragraph should already be inserted at line 143/144, before presenting the overly speculative final conclusion/suggestion.

We agree with this comment and moved this part to the beginning of the discussion.

25. Line 164 again mentions “inhibition of calcification”, whereas the measure values are not zero. A more “nuanced” wording is absolutely necessary!

Agree, see response to comment 19 and 22 (inhibited is replaced with "severely reduced")

26. Line 166: “Moreover, the Symbiodinium symbionts clearly exhibit stress earlier than the host.” True, that is to say, for the indicator you use, i.e., calcification rate. However, this may not be the best indicator. Maybe the host would show stress just as early (or even earlier) if you would use another indicator (e.g., locomotion, feeding behaviour, reproduction, etc.). And finally, with symbionts showing signs of stress, it is hard to image the foram itself is not “feeling” signs of stress!

I simply want to underline that in my opinion you can't reduce the “well-being” of the foram to its calcification efficiency. This is only one element out of many others, which may not even be critical!

See response to comment 2.

27. Lines 166-67: “The different thermal sensitivity of the symbionts and host of *S. orbiculus*”. Same remark here. You can't base your ideas on the “thermal sensitivity of the host” (= the whole holobiont) only on its calcification rate. I would say that the thermal sensitivity of *S. orbiculus* depends both on the thermal sensitivity of its calcification rate, on the thermal sensitivity of its symbionts and on the thermal sensitivity of many other of its life processes.

I think you should rather write: “The different thermal sensitivity of the calcification rate and of the symbionts of *S. orbiculus*”.

Agree. We have changed this sentence as suggested.



28. Line 168-69: “Hallock et al., 2006b which (=who) suggested that the ectoplasm of bleached specimens is “preprogrammed” to continue calcification.” → This sentence needs some more explanation!

This notion was given by Hallock et al., 2006b to explain their own observations on bleaching in *Amphistegina*.

29. Lines 169-70: “Our observation of *S. orbiculus* indicates that this discordance might be limited to a relatively short time after the bleaching”. → I have no idea what you are talking about! What “discordance” do you mean? (probably the wrong word!). What can your observations on *S. orbicularis* tell us about bleaching? I’m lost! Please clarify!

We removed these sentences.

#### Conclusion

30. Lines 187-88 “Our study emphasizes the role of pre-exposure and acclimation processes in mitigating the effect of future warming.” It is very strange to me that this point, which is only discussed very briefly at the end of the discussion, suddenly becomes the main conclusion of your work!

Agree. We removed this notion from the conclusion.