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Interactive comment on "Foraminiferal holobiont thermal tolerance under climate change – Roommates problems or successful collaboration?" *by* Doron Pinko et al.

Anonymous Referee #2

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General comments:

The study presented by Pinko et al. represents a comparative study of two different common LBF species, with different shell and symbiont types, exposed to elevated temperature over few weeks. The two main proxies assessed give insights into photosymbiont performance and holobiont health. Due to subtle differences, the authors conclude that Sorites orbiculus will be less affected by climate change than Amphistegina lobifera. They also claim insights into distinct effects of pre-exposure to moderate temperatures regarding the LBFs thermal tolerance. Along the lines of former studies, the experiment shows that there are species-specific thresholds regarding tempera-

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ture and duration of exposure, and that LBF from the Eastern Mediterranean, which are most likely Lessepsian invaders from the Red Sea, have a relatively high thermal tolerance. The further confirm that the photosymbionts seem to be the 'weaker' member of this symbiotic association, showing the earlier stress response. Hence, the study give further important proof of prior hypotheses on LBF thermal stress responses, and adds to the knowledge of species-dependent thresholds. Hence, I consider it important and valid to publish this data. However, the novel insights are limited, as I do not think that calcification can be considered as a host-specific response (as they suggest), and therefore this study does not assess the relative contribution of host and symbionts (see specific comments).

Specific comments:

-Calcification cannot be considered as a host proxy, as it is largely influenced by photosynthesis. It is hence, as in many other studies, a holobiont proxy. Although prior studies mostly used possibly less precise methods to assess growth (e.g. increase in surface area or buoyant weight in studies by Schmidt, Prazeres, Stuhr, Hallock and others), it basically gives similar information. Nearly all studies on LBF stress response assessed at least one holobiont parameter such as growth (often also others to get a better picture, as calcification / growth can be limited due to other factors that are independent of stress, hence, it is not a very good parameter anyways), and one or more photosymbiont parameters. The only study to my knowledge that actually managed to gain host-specific insights was Stuhr et al. 2018 (Scientific Reports) by differentiating between host and symbionts on the protein level. But even here, the influence of photosymbionts stress on host stress cannot be fully excluded.

- Due to the lack of novelty described above, I would suggest to the authors to focus more on the comparison between A. lobifera and S. orbiculus, and the detected differences in time-related responses (seasons and experimental duration), and emphasize these in more detail.

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- Furthermore, the methods section is very short and lacking a lot of details, descriptions etc., and many crucial information has unfortunately been moved to the supplementary materials. The same applies to some of the results, e.g. the statistics, which should be at least indicated in the text or the figures where significant. Even with the results provided in the supplementary materials, it is not possible to fully judge where statistically significant variation were detected due to the poor representation and lacking explanations.

- I am also wondering why no further parameters were tested, and calcification and photosynthesis normalized by individual, which is very unusual and prohibits comparison with other studies. It is also less precise if not all individual were of the same size (which they most likely were not). And I wonder, if you measured photosynthetic activity via oxygen production, why didn't you simply also measure respiration via oxygen consumption in darkness? This would have provided another valuable indicator for holobiont condition, and would have allowed to calculate gross oxygen production. So far, you only provide net values for photosynthesis, not considering that respiration is likely to be higher under high temperatures, which results in lower net photosynthesis, even though the actual production of oxygen may be constant.

- Lastly, I think this study could further be improved by discussing some interesting observational details such as bleaching (Did you observe it? In which species? Mottled or more gradual? Or mortality? Reproduction?) as well as by including time into the statistical evaluation of the results (e.g. two-factorial analysis of variance with time and temperature as factors).

Technical comments:

L2: "Roommate problems or successful collaboration?" this title sounds catchy, but the question is still as valid as before... I don't think the paper is resolving this question, and also the first part of the title is very broad (e.g. climate change includes more than just temperature stress) and should be a bit more specific

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L11: "... hyaline diatom-bearing Amphistegina lobifera and the proceallaneaous dinoflagellate-bearing Sorites ..."

L12-13: see discussion above

L16-L17: "future warming will significantly shift the relative contribution..." this is taking the implications way too far. You only see small differences in their response to 35°C in respect of timing. Please be more specific (in general) and stick to what you actually show.

L18-21: You mention pre-exposure for the first time here, and it is rarely described in the manuscript in general. What do you mean by this? The season? Or different acclimation temperatures? I also don't understand why you suggest that it reduces thermal tolerance. Please reconsider these statements and adjust to your results and the discussion.

L27-28: "... one of the regions most affected..."

L30: "Symbiont-bearing large benthic foraminifera ..."

L34-35: To my knowledge, none of these studies really provided evidence for temperature control on symbionts composition. Some suggested that there may be a connection, but statements saying that they are "strongly controlled" would definitely require further proof, especially since other studies did show extremely flexible relationships (e.g. several Lee et al. studies, Pochon et al. 2007, Schmidt et al. 2016)

L45: delete "calcifiers"

L55-56: there are much earlier studies that describe these species in much more detail (such as Hansen & Burchard 1977 and Hottinger 1977) that deserve to be cited here.

L59: "... Israel, during..."

L60: the picked size fraction is crucial when it comes to assessing growth/calcification as it is strongly linked to ontogenetic phase. Hence, please provide this information in

the manuscript and not in the supplemental materials.

L62: the same accounts for the sample sizes. It is important to know in order to judge the power of the study. And "... 60-ml airtight..."

L 68: there is still a lot of detail missing regarding the culture conditions: was there water flow? What kind of water were they in? Were they fed? pH? ...

L65: Which temperatures? In the baths or the flasks? What means regularly? Once a week or once per hour would both be regularly but are very different...

L71: Why did you use calcification rate as the only parameter? Please explain.

L74: Same for: why did you only include A. lobifera for the spring experiment?

L77: Which "constant conditions"?

L79-80: How many samples didn't show oxygen production? And any suggestion why?

L81: How was temperature adjusted? What instruments did you use to control this?

L82: where are these temperatures expected? In the Med Sea? The Red Sea?

L85: Was the water filtered? Pre-conditioned temperature-wise?

L85-90: Please give more details on the method. Did you do this in the 60-ml flasks? What was the time frame? Were foraminifera pooled?

L91-93: Same details are missing here, as well as references. What instruments did you use? What light? Which temperatures? "... μg L-1 ..." and please define RDO. What was the accuracy value? And in general, you normalize both parameters by specimen. For better comparability, they are usually normalized by species size (given by surface area or weight etc.). Please consider doing so.

L95-97: Please give n for each parameter, treatment, time point, described which data got transformed, and which tests exactly got chosen "accordingly".

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L99: " . . .cases where normality . . . non-parametric Kruskal-Wallis test . . ."

L100: Please name the "proper" post-hoc tests.

L106: you cannot say whether there would be differences in Al. lobifera between winter and spring, but sounds as if you do. Please rephrase.

L108: What is the "x" in your box plots? Please indicate significant differences by a letter report. And give n in captions. Also provide full species names and specify what the whiskers represent (SE?).

L112&L130: keep descriptions consistent

L113f: Please avoid expressions like "much higher", "a decrease" or "substantially different". What does that mean? Please provide statistical test results and/or how big is the difference (twice as high, ∼20 %...)

L120: "The symbionts' photosynthetic . . ." and how is the "sensitivity pattern" different? Apart from one week in the 35°C treatment, they look very similar to me.

L122: As mentioned before, please provide overview of statistical results here, ideally in figure.

L123: If you mention this "abnormal value" please state in which way it was abnormal and why you suspect this to be related to handling. You say it's not used for average and SE calculations. Does that also mean the further statistical analyses?

Fig. 3: please jitter weeks more, so they are easier to recognize. In which week did you have the extreme value?

L128: "significant negative response" I am not sure the word significant is used correctly here.

L134: "week" and what means "massive bleaching"? Please describe, give proportions etc.

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L135-136: "... between 25°C and 32°C, and was thus clearly ..." As described in the general comments, please describe it more. What may be the reason for this bleaching? Were both species affected? A. lobifera could have a lower light tolerance than Sorites and could therefore bleach. Or since it only affected the lower temperature range, it could actually be related to reproduction. Why didn't you exclude the bleached specimens from the analyses? I suggest to include the data anyways, e.g. only for comparison in the supplementary, or to conduct analyses on those samples that had no bleaching. Moreover, as the 35°C had no bleaching, please provide at least these values to compare with former weeks.

L142-143: Well, I think "clear differences in thermal tolerance" is a bit exaggerated. I would call them rather subtle.

L144: This is not true, the calcification seems lower at 35°C (significances missing), at least in the winter populations. Plus, the experimental exposure in spring was shorter, so the response may have just been delayed, as suggested by the reduced oxygen production.

L147: please rephrase, something here doesn't make sense. And I again don't agree that you can state that there is a "strong dependence", as many other factors have been shown to be at least as important. Please also include some newer references here.

L149&L161: "dinoflagellates" and change to "Symbiodinium" to Symbiodiniaceae (as you seem to be aware this taxonomic system has been revised)

L153: "... control a holobionts ..."

L154-155: What "mechanism to cope with thermal stress was observed"? Please describe, I do not think this paper actually showed 'shuffling'.

L156: "... explain the observation ..."

L159: "... describes ..."

L164: “. . . 35°C, whereas in . . .”

L165: “. . . inhibited, indicating that it is. . .”

L166: That means you refer to Sorites only? When is “earlier”?

L168: An apparent higher sensitivity (earlier/stronger response of the symbionts than the holobiont) was also observed by other studies such as Prazeres et al. 2017, Stuhr et al. 2017, Schmidt et al. 2016. . .

L170: Here you mention bleaching again: so were these specimens that you measured calcification on already bleached? If there was, this may also indicate that there was another stress factor such as too high light intensity or the wrong light spectrum (e.g. Hallock once showed that blue light facilitated high growth rates but at the same time led to bleaching). Please discuss your observations.

L172: The resolution is a very important point! One week is a long time for a foraminifera! Plus, so far I don’t even know how long your calcification measurements or photosynthesis measurements took. They don’t calcify continuously all day long, so the time frame may strongly influence the results. The same accounts for the photosynthesis, which varies over the time of the day.

L174: Please specify the time of the onset.

L175: What do you mean by “very cold”? I think that is very relative. . . give a temperature range of what is usually encountered in the Med Sea in winter and spring, and ideally state what were the temperature measured during sampling in the methods section.

L178: “. . . symbionts, or both. However, while the A. lobifera spring . . . “

L180: In which way did they respond “negatively”? Please be a bit more specific so the reader does not have to go back to each of the studies you cite to find you what you mean, and “. . . that, while . . .”

L181: “. . . temperatures benefits the . . .“

L186-187: There have been studies modeling the future changes in distribution and hence contribution, which should be cited here (e.g. Weinmann et al. 2013, 2017)

L187: If you mention pre-exposure here (as in the abstract) please elaborate a bit more in which way they had different pre-exposures in the methods as well as the discussion parts.

L188-189: I don’t understand this statement at all. Why? Where is you evidence for that?

L189: Again, I find “clearly shows” a bit exaggerated. Supplement 1: Is the number of replicates given the value before or after exclusion of same samples? Why are the numbers different for the different time points? How did you deal with this unbalanced design in your statistical analyses?

You used once filtered and once unfiltered water. Why? And why did you pre-condition the spring Sorites to another temperature than the rest? This is very crucial information and must not be excluded from the actual manuscript! I am not sure if you can compare your data the way you do with all these differences.

Sometimes you give two numbers after the comma, sometimes six or other. . . please be consistent (and usually its three).

Why do you give four stars (they are actually called asterisk)? Usually, these are used to indicate the level of significance, from one (lower end of significance) to three (highly significant).

Why is some text red? Please explain in captions.

Table S2.2 and others: What are “1, 2 and 3” in your column headers? I cannot understand your statistical results if I don’t know what is which group.

Put spacing equally before and after “=”, but not after “(“.

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Table S5.2: “Stars indicate homogenous groups and thus significant differences between them”? This makes no sense to me, because if they are homogenous, they are similar, so no difference. . .

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