Foraminiferal holobiont thermal tolerance under future warming - Roommates problems or successful collaboration?

Doron Pinko, Sigal Abramovich, and Danna Titelboim

The Department of Geological and Environmental Sciences, Ben-Gurion University of the Negev, Beer Sheva, 8410501, Israel.

Correspondence to: Danna Titelboim (dannati@post.bgu.ac.il)

Abstract

Understanding the response of marine organisms to expected future warming is essential. Large Benthic Foraminifera (LBF) are symbiont bearing protists considered to be major carbonate producers and ecosystems engineers. We examined the thermal tolerance of two main types of LBF holobionts characterized by different algal symbionts and shell types (resulted from alternative biomineralization mechanisms): The hyaline diatom-bearing *Amphistegina lobifera* and the porcelaneous dinoflagellate-bearing *Sorites orbiculus*. In order to assess the holobiont thermal tolerance we separately evaluated foraminiferal calcification rates and symbionts net photosynthesis under present-day and future warming scenarios. Our results show that both holobionts exhibit progressive loss of life functions between 32ºC and 35ºC. This sensitivity differs in the magnitude of their response: calcification of *A. lobifera* was drastically reduced compared with *S. orbiculus*. Thus, future warming may significantly shift the relative contribution of the two species as carbonate producers. Moreover, *A. lobifera* exhibited a synchronous response of calcification and net photosynthesis. In contrast, in *S. orbiculus* the symbionts decreased net photosynthesis prior to calcification. This implies that algal symbionts are limiting the resilience of the halobiont.

1 Introduction

Since the beginning of the industrial revolution anthropogenic activity has been leading to rapid ocean warming. This negatively affects marine ecosystems and specifically symbiont bearing calcifiers (Kawahata et al., 2019). The observed rate of global Sea Surface Temperature (SST) rise stands on 0.11ºC per decade and future scenario predicts a similar rate until the end of the century (IPCC, 2014). Therefore, the Mediterranean can be presented in biogeographic models as a "miniature ocean" providing indications on global patterns in marine ecosystems in a warmer world (Lejeusne et al., 2010). Warming in the Eastern Mediterranean is expected to rise almost four times more rapidly than global forecast (Macias et al., 2013). Thus, the Eastern Mediterranean is expected to be one of the regions most affected by global warming.

Symbiont-bearing Large Benthic Foraminifera (LBF) are single-celled ecosystems engineers. Their carbonate production is estimated as at least 5% of the annual production in reef and carbonate shelf environments (Langer, 2008; Langer et al., 1997). Temperature is a major factor in the distribution of LBF that exhibit distinct thresholds for reproduction, survival, bleaching,

and calcification (Evans et al., 2015; Hallock et al., 2006a; Langer et al., 2012; Langer and Hottinger, 2000; Schmidt et al., 2011; Titelboim et al., 2019; Weinmann et al., 2013). The symbiont composition of LBF was suggested to be controlled by temperatures (Momigliano and Uthicke, 2013; Prazeres, 2018; Prazeres et al., 2017; Prazeres and Renema, 2019; Schmidt et al., 2018) which explains the observation that species-specific thermal tolerance is associated with more diverse algal symbionts (Stuhr et al., 2018).

Many LBF species are Lessepsian invaders, which often comprise over 90% of the foraminiferal population in the Eastern Mediterranean (Hyams-Kaphzan et al., 2014; Titelboim et al., 2016). Their invasion and successful establishment are facilitated by rising temperatures, as in the case of other Lessepsian organisms (Por, 1978, 2010; Zenetos et al., 2010, 2012). However, some of these species currently live very close to their upper thermal thresholds and consequently, their presence will be impeded in the relatively near future (Titelboim et al., 2016). The thermal sensitivity of some LBF species has already been investigated (Schmidt et al., 2011, 2016b; Stuhr et al., 2018; Titelboim et al., 2019). Yet, the relative contribution (positive or negative) of the holobiont components to cope with rising temperatures has not been fully constrained.

In this study, we present the thermal sensitivity of two very dominant and prominent LBF holobiont systems (Fig. 1). Specifically, our study separately assesses the thermal sensitivity of the foraminiferal host calcification rate and algal symbionts net photosynthesis as an indication of their well-being under different warming scenarios. This approach was chosen since calcification is a physiological activity done only by the foraminifera and thus presents a proxy to its wellbeing (like many organisms, when stressed lowering physiological activities that involve high consumption of energy). The same is true for photosynthesis, which is a primary life process and thus present an efficient indicator for the tolerance of the symbiont algae. Because of the exclusiveness of each parameter we could use them to disentangle the complex relationship between the two components of the holobiont.

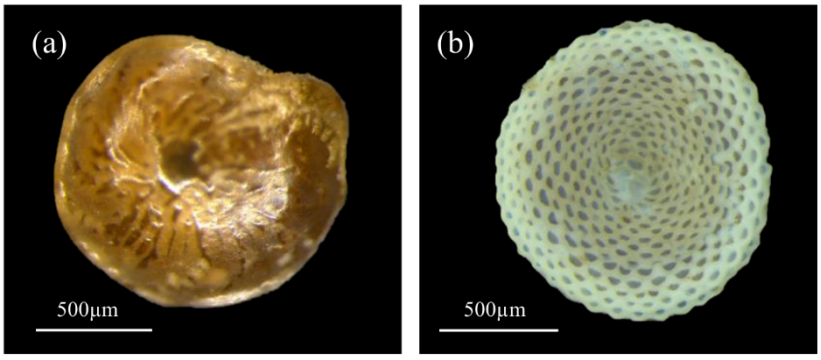

Figure 1: The holobionts examined in this study. a) *Amphistegina lobifera* and b) *Sorties orbiculus*. Note the green-brownish color of the symbiont algae.

2 Materials and methods

2.1 Specimens collection and handling

In this study, we targeted two LBF species that represent different types of holobiont systems, which differ in their shell construction mechanism and algal symbionts: *Amphistegina lobifera* (diatom bearing hyaline, Larsen, 1976, Prazeres et al., 2017; Schmidt et al., 2015, 2016b) and *S. orbiculus* (dinoflagellate bearing porcelaneous, Merkado et al., 2013; Pawlowski et al., 2001; Pochon et al., 2014). Both species have cosmopolitan distributions, are very common in warm shallow marine environments (Langer and Hottinger, 2000) and display different thermal tolerances (Titelboim et al., 2016). Specimens were picked from macro-algal samples that were scraped from beach rocks at Shikmona, northern Mediterranean coast of Israel. To reduce variance in growth derived from ontogenetic variability, the specimens were picked between the specific size fractions of 750-1000 μm. Live specimens (indicated by their symbiont color and motility) were cleaned by brushing, divided into groups with an equal number of of specimens (40 *S. orbiculus* and 30 *A. lobifera*), and transferred into 60-ml airtight Erlenmeyer flasks filled with natural seawater filtered to 0.45 μm, from here on referred to as 'samples'.

2.2 Laboratory manipulative experiments

We conducted temperature manipulative experiments on *S. orbiculus* and *A. lobifera*. In these experiments, the well-being of both holobionts was examined by separately determining the responses of the foraminiferal calcification rate and symbiont algae net photosynthesis to elevated temperatures. These are both very accurate quantitative parameters. As such, they were chosen for this study that aimed to recognize even subtle differences between treatments and between species.

During the experiments, the samples were placed in temperature-controlled water baths, which maintained constant temperatures of ± 0.5°C, temperatures were monitored using HOBO data loggers that recorded temperature every one hour. During the cultivating period, the samples were kept under a daily cycle of 12 hours light / 12 hours dark using fluorescent light of ~30 μmol photons m^{-2} s^{-1}. These are lower than the photosynthetic optimum for *A. lobifera* (Ziegler and Uthicke, 2011), However, using these light conditions, we were able to produce data comparable to those presented in related published papers (Schmidt et al., 2016b, 2016a, 2018; Titelboim et al., 2019). These light levels should not cause stress since LBF acclimate rapidly to different light levels (in under 48 hours, Ziegler and Uthicke, 2011) and thus the 10 days acclimation is sufficient for them to adjust to the specific light level provided during the experiment.

All samples were acclimated under constant conditions for at least ten days. Acclimation temperatures were optimal for each species: 27ºC for *S. orbiculus* and 25ºC for *A. lobifera* and other conditions are as described in 2.1. Then, the calcification rate and net photosynthesis were measured to establish the performance baselines of the different species and the natural variability between samples, under equal conditions. Two samples (one *A. lobifera* replicate from 25ºC treatment and one *S. orbiculus* replicate from 30ºC) did not exhibit similar values of net oxygen production as the majority of samples and were excluded from the rest of the study to avoid bias. At the end of the acclimation period, seawater was replaced in all samples and the temperature of each bath was slowly adjusted (1°C/hour). The examined treatments (25°C, 30°C, 32°C, 35°C) represent current

and future temperatures expected in the Eastern Mediterranean until the end of the century (Macias et al., 2013). Each temperature treatment included four replicates unless reduced to three following low performance of the symbionts (*A. lobifera* 25ºC and *S. orbiculus* 30ºC). After acclimation, following each week, the water was replaced with fresh natural seawater with verified pH of 8.0-8.1 and salinity of 38.4-39.2. The replaced water from all the samples was transferred to air-tight syringes and then all oxygen samples were immediately measured. Alkalinity measurements were conducted over the next two days. To ensure no changes accurse in this time frame standard material was measured before and after the first and last sample of the set, respectively. Calcification rates (µmol $CaCO_3$ $week^{-1}$ $specimen^{-1}$) were calculated using the Alkalinity Anomaly Method (Smith and Key, 1975). In this method, the calcification rate is determined from the change in total alkalinity of the seawater caused by the precipitation of $CaCO_3$. These are determined by comparison to a control sample containing no foraminifera. Accuracy was assessed by analyses of the Scripps Institute of Oceanography reference seawater (Batch 180) and an internal standard. Calcification rate involves high energetic consumption and as such is drastically influenced by stress levels of a calcifying organism and was specially shown to be related to thermal stress in benthic foraminifera (Evans et al., 2015, Schmidt et al., 2016b, Titelboim et al., 2019). Net photosynthesis (ΔO_2 µg L^{-1} $specimen^{-1}$) was measured as net oxygen production compared with a control sample containing no foraminifera. Dissolved oxygen was measured using Eutech DO 450 connected to a Rugged Dissolved Oxygen (RDO) sensor. Accuracy was assessed by calibration of the sensor against Winkler titration.

2.3 Statistical analysis

To examine whether differences in calcification rates and net photosynthesis are significant between temperature treatments and between weeks, statistical analyses were performed using STATISTICA10 software. For each set of data, we tested assumptions of normality of the residuals and homogeneity of variances and a statistical test was chosen accordingly. If both assumptions were valid ANOVA was performed, in cases where normality was valid and homogeneity was violated Welch's ANOVA test was applied. In cases where normality was violated the non-parametric test Kruskal-Wallis was applied. Each was followed by the proper post-hoc test. All statistical analysed are summarized in table 1.

Table 1: description of all statistical analyses conducted in this study including which statistical test was preformed, if data was transformed, and the number of samples in each data set.

Data		Description	Number of samples	Statistical analysis
Baseline	Calcification rate	Comparison between *S. orbiculus* and *A. lobifera* after acclimation period	*S. orbiculus*: 15 *A. lobifera*: 14	1-way ANOVA on log-transformed data
	Net photosynthesis	Comparison between *S. orbiculus* and *A. lobifera* after acclimation period	*S. orbiculus*: 15 *A. lobifera*: 15	1-way ANOVA on log-transformed data
Sorites orbiculus	Calcification rate	Comparision between 4 temperatures and 3 weeks	15	2-way ANOVA and Tukey HSD test

	Net photosynthesis	Comparison between 4 temperatures	Weeks 1&2: 15 Week 3: 14	Kruskal Wallis test and Multiple comparisons
		Comparison between the 3 weeks		1-way ANOVA
Amphistegina lobifera	Calcification rate	Comparison between 4 temperatures and 2 weeks	15	2-way ANOVA on log-transformed data and Tukey HSD test.
	Net photosynthesis	Comparison between 4 temperatures and 2 weeks	15	2-way ANOVA and Tukey HSD test
	Third week bleaching of *A. lobifera*	Comparing the number of bleached specimens between 4 temperatures	15	1-way ANOVA on log-transformed data and Tukey HSD test.

3 Results

Our experimental design takes into consideration biological variability in calcification rates and net photosynthesis between different species. This notion is based on previous observations that different species even from the same genus, and different populations of the same species display different calcification rates under the same conditions (i.e. baseline, Titelboim et al., 2019). Specifically, among our experiments the activity baseline of both calcification and net photosynthesis are significantly different between *A. lobifera* and *S. orbiculus* (One-way ANOVA: p value < 0.001, Fig. 2, Supplementary Tables S1 and S2). Hence, the thermal tolerance of the two holobionts was separately evaluated for each experiment.

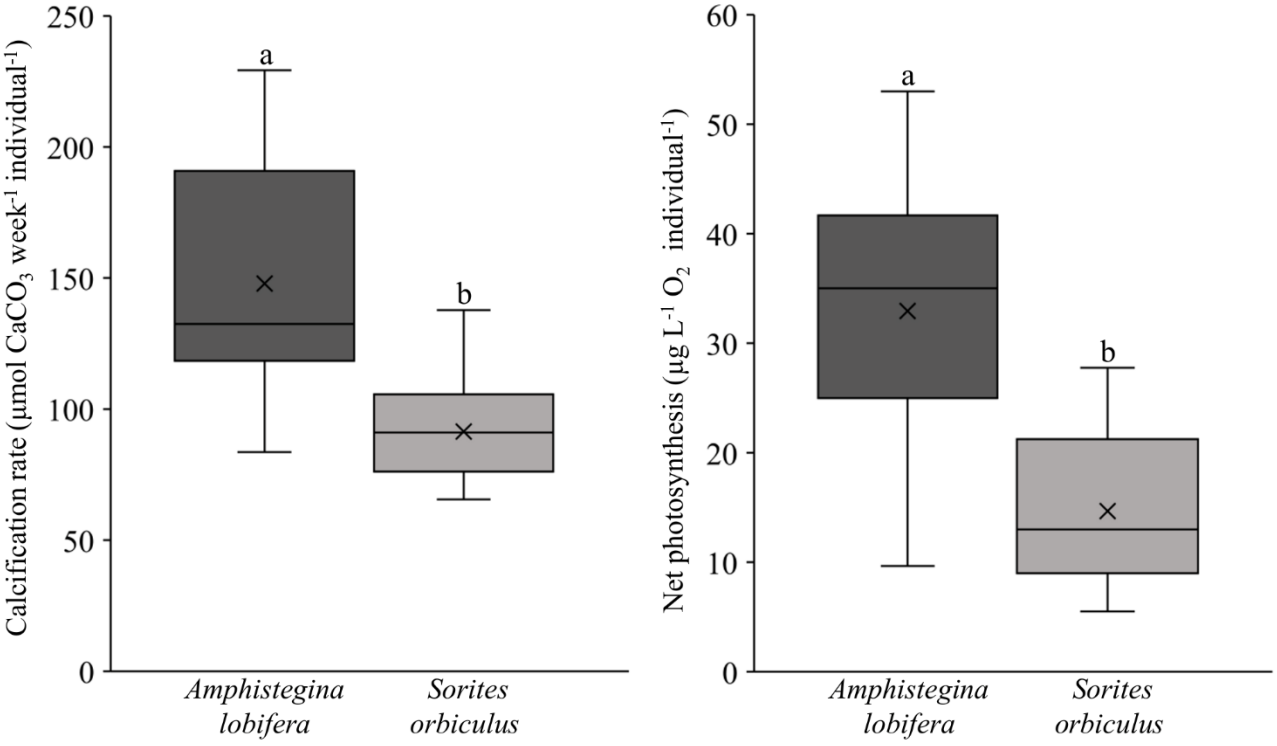

Figure 2: Activity baseline of the foraminiferal calcification rate (left) and symbionts net photosynthesis (right) of *A. lobifera* (n = 14, 15) and *S. orbiculus* (n = 15, 15) . Note, the significant differences in baseline values of both calcification rates (p <0.001) and photosynthetic activity (p <0.001) between the two holobiontsError Bars represent minimum and maximum values.

3.1 *Sorites orbiculus* (porcelaneous- dinoflagellate holobiont system)

Calcification rates of *S. orbiculus* under the different temperature treatments exhibited highest values at 25°C, 30°C, and 32°C. A mild not statistically significant decrease was observed at 35°C (Fig. 3, Supplementary Tables S3.1 and S3.2). Net photosynthesis s show positive values under 25°C, 30°C, and 32°C. At 35°C, net photosynthesis was negative (Fig. 3, for statistical analyses, see Supplementary Table S4.1 and 4.2). Unaccountably, in week 3 one sample exhibited an abnormal high value (i.e. extreme in Fig. 3) with respect to previous weeks as well as to other replicates and thus was not included in the average and error calculations nor in the statistical analysis.

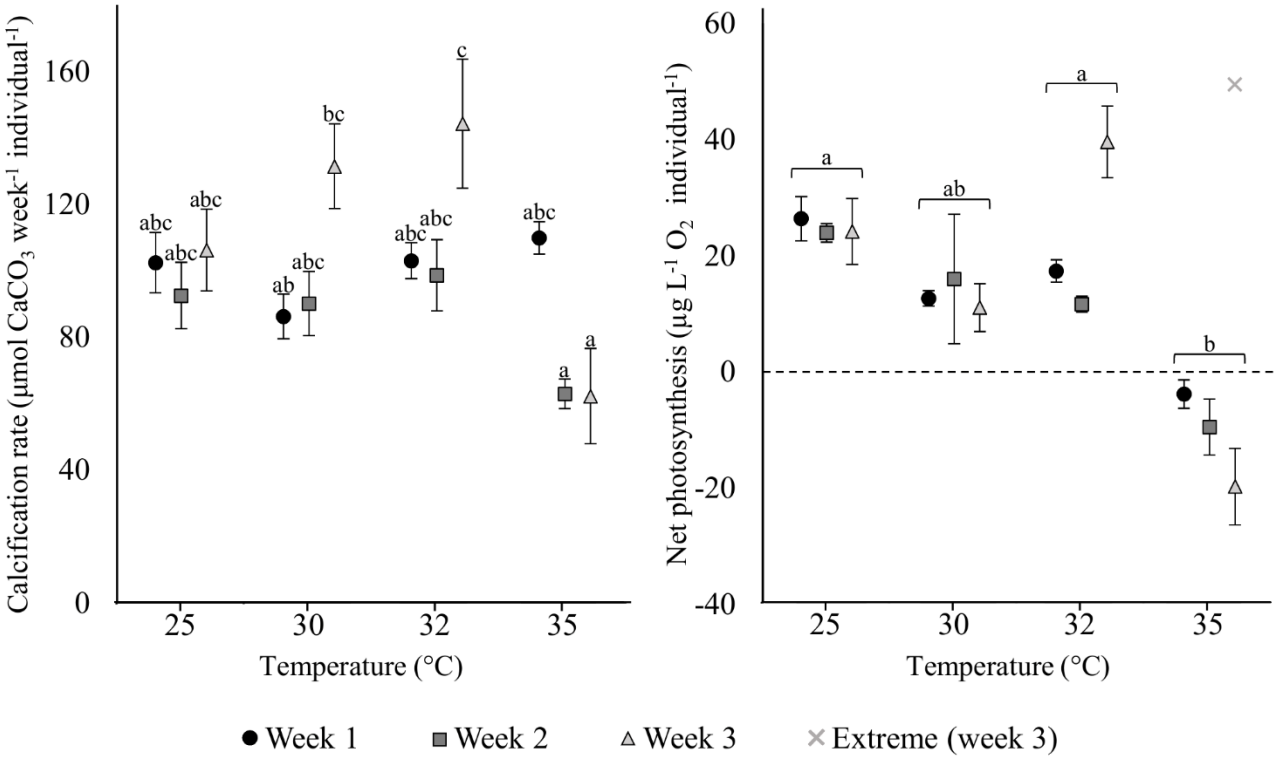

Figure 3: Calcification rates (left) and net photosynthesis (right) of *S. orbiculus*. Letters represent the results of the post hoc tests (Supplementary Tables S3.2 and S4.2). Error bars are SE. A single abnormal measurement, obtained at week 3 is marked as extreme and is not calculated as part of the average, error, and statistical analysis.

3.2 *Amphistegina lobifera* (hyaline diatom holobiont system)

Both calcification and net photosynthesis are synchronous throughout the experiment. After the first and second weeks, calcification rates and net photosynthesis exhibited the highest values under 25ºC, 30ºC, and 32ºC. At 35ºC calcification and net photosynthesis were both severely reduced and net photosynthesis was negative (Fig. 4, Supplementary Tables S5 and S6). Between the second and third weeks, many specimens exhibit massive bleaching that occurred in different treatments between 25ºC-32ºC in similar proportions (Tukey HSD post-hoc test, p values> 0.1, Supplementary Table S7.2) and thus was clearly not related to the different temperature. Bleaching in the 35ºC treatment did not exceed 2 specimens per replicate (Supplementary Table S11). For this reason, measurements of the third week are excluded from the results.

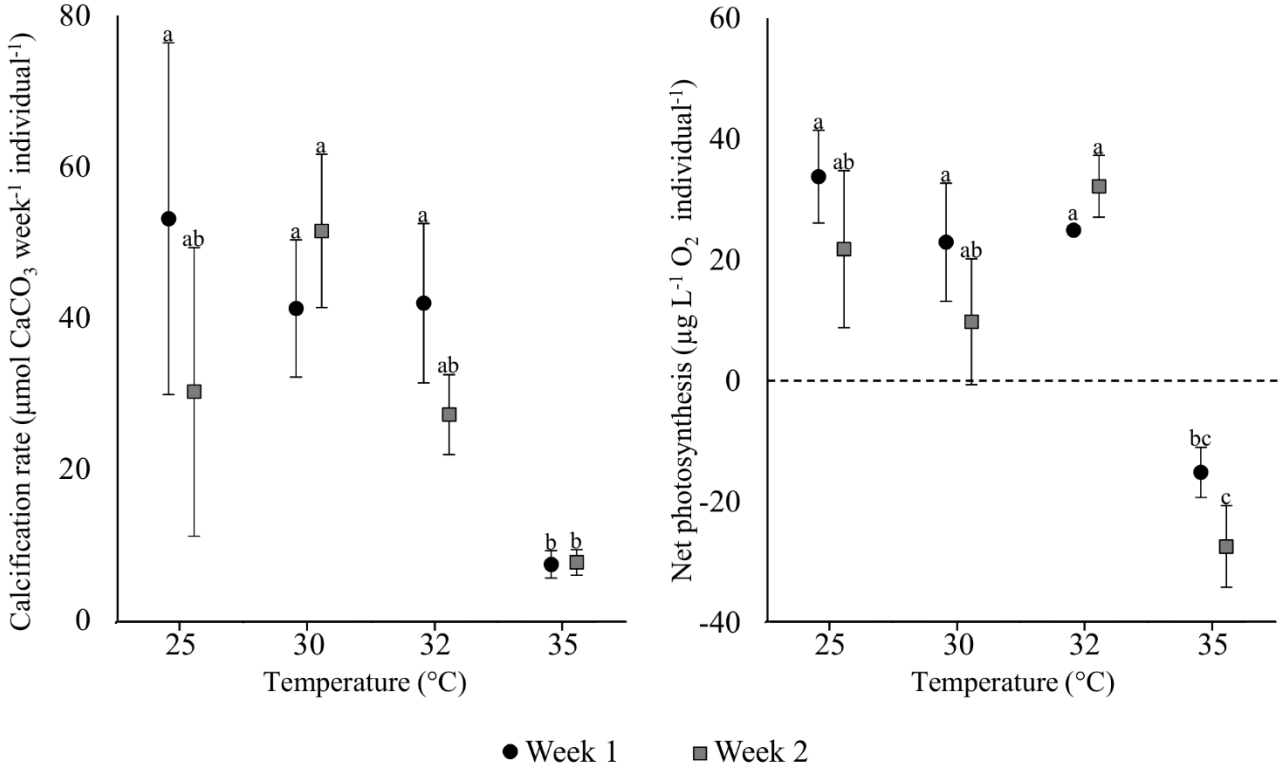

Figure 4: Calcification rates (left) and net photosynthesis (right) of *A. lobifera*. Note the synchronous negative response at 35ºC. Error bars are SE, and letters represent the results of the post hoc tests between temperatures and weeks (Supplementary Tables S5.2 and S6.2).

4 Discussion

Our study separately describes the thermal sensitivity of the foraminifera and the algal symbionts in two types of holobiont systems: *A. lobifera* hosting diatoms mostly from the order Fragilariales (Barnes, 2016; Prazeres et al., 2017; Schmidt et al., 2016b, 2018) and *S. orbiculus* hosting dinoflagellates, Symbiondiniaceae (Merkado et al., 2013; Pawlowski et al., 2001; Pochon et al., 2007). Both species are considered as prominent calcifiers based on their massive occurrences and widespread distribution (Langer and Hottinger, 2000) and both record a graduate decline in physiological performance between 32ºC to 35ºC (Figs. 3-4). Both holobionts show thermal resilience up to 32ºC and a negative response at 35ºC (Figs. 3 and 4). Yet, they differ in respect to the magnitude of their responses: *A. lobifera* and its diatom symbionts share similar thermal sensitivity with near inhibition of calcification and negative net photosynthesis at 35ºC, whereas in *S. orbiculus* calcification is less dramatically reduced at 35ºC, indicating it is more resilient to extreme SST than *A. lobifera*. Moreover, the Symbiondiniaceae symbionts exhibit stress earlier (already after the first week) then calcification that was not reduced at the first week and only slightly reduced after. The different thermal sensitivity exhibited by calcification rate and by symbionts of *S. orbiculus* imply that they might be a limiting factor for the host to cope with future warming. A similar apparent discordance was previously observed in *Amphistegina* (Prazeres et al. 2017, Stuhr et al. 2017, Schmidt et al. 2016 and Hallock et al., 2006b). Hallock et al., 2006 suggested that the ectoplasm of bleached specimens is "preprogrammed" to continue calcification. Possible explanations for the synchronized response of the *A. lobifera* holobiont in this study are either 1) similar thermal sensitivity of the symbiont and the host or 2) the weekly resolution of measurements may not capture a short discordance time between the responses of the symbiont and host.

It was previously shown that corals ability to cope with elevated temperatures is related to their partnering with functionally diverse symbionts (Baker et al., 2004; Howells et al., 2012; Jones et al., 2008; Poquita-Du et al., 2020; Rowan, 2004) although their symbiosis is limited to dinoflagellate from the *Symbiondiniaceae "*Clades*"* (LaJeunesse et al., 2018; Silverstein et al., 2015). LBF are known to host different kinds of symbionts (Pochon et al., 2007), which include dinoflagellates, diatoms, unicellular chlorophytes, unicellular rhodophytes and/or cyanobacteria (reviewed in Lee, 2006). Whereas the general types of the symbiont (algal genus) seem to be phylogenetically fixed, there appears to be considerable flexibility in symbiont infestation, even within one individual (Lee, 2006). This versatile symbiont partnership may control the holobionts thermal tolerance and provide one of the key factors in their response to future warming. For example, a mechanism to cope with thermal stress was suggested in *Pararotalia calcariformata*, an extremely heat tolerant symbiont bearing foraminifera, that host a diverse symbiont community of diatoms. In case of thermal stress, functionally relevant members of the symbiont community can become more dominant and magnify the ability of the holobiont to tolerate elevated temperatures (Schmidt et al., 2018). This might also explain the observation that species-specific differences in the thermal tolerance of *Amphistegina* species are correlated to different symbiont assemblages. Specifically, a larger diversity of algal symbionts was associated with the more tolerant species (Stuhr et al., 2018).

Conclusions

Considering the role of LBF in the carbon cycle and as ecosystem engineers, their future with expected warming is a major concern. Previous study modelled the predicted changes in the distribution of LBF and their contribution to carbonate production (e.g. Langer, 2008; Langer et al., 2013; Weinmann et al., 2013; Weinmann and Langer, 2017). However, our results highlight the need for species-specific considerations for more accurate predictions on the fate of LBF.

Our study shows that LBF have different thermal tolerances that are limited by the sensitivity of their eukaryotic algal symbionts. Considering recent findings on the significant role of the prokaryotic microbiome on the physiological performance of LBF (Prazeres, 2018; Prazeres et al., 2017), it will be highly valuable also to explore in future studies their specific contribution to the thermal tolerance of the holobiont.

Data availability

All data related to the manuscript is available in the Supplement .

Author contribution

The study was designed by D.T. and D.P. Sampling and culturing experiments were carried out by D.P and D.T. using facilities provided by S.A.; Interpretation of data and writing of the manuscript were done by all authors: D.P., D.T., and S.A.

Competing interests

The authors declare that they have no conflict of interest.

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