



# Foraminiferal holobiont thermal tolerance under future warming – roommate problems or successful collaboration?

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**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 ecosystem engineers. We examined the thermal tolerance of two main types of LBF holobionts characterized by different algal symbionts and shell types (resulting from alternative biomineralization mechanisms): the hyaline diatom-bearing *Amphistegina lobifera* and the porcellaneous-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 32 and 35 °C. 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 limit the resilience of the holobiont.

## 1 Introduction

Since the beginning of the industrial revolution, anthropogenic activity has led 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 at 0.11 °C per decade, and future scenarios predict 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 of global patterns in marine ecosystems in a warmer world (Lejeune et al., 2010). Warming in the Eastern Mediterranean is expected to rise almost 4 times more rapidly than the 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 ecosystem engineers. Their carbonate production is estimated to be 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 exhibits 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; Weimann et al., 2013). The symbiont composition of LBF was suggested to be controlled by temperature (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). However, 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 symbiont 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 represents a proxy of its well-being (like many organisms, lowering physiological activities that involve high consumption of energy when stressed). The same is true for photosynthesis, which is a primary life process and thus presents 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.

## 2 Materials and methods

### 2.1 Specimen 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 *Sorites orbiculus* (dinoflagellate-bearing, porcellaneous; 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, on the 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 and 1000  $\mu\text{m}$ . Live specimens (indicated by their symbiont colour and motility) were cleaned by brushing, divided into groups with an equal number 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  $\mu\text{m}$ , hereafter referred to as “samples”.

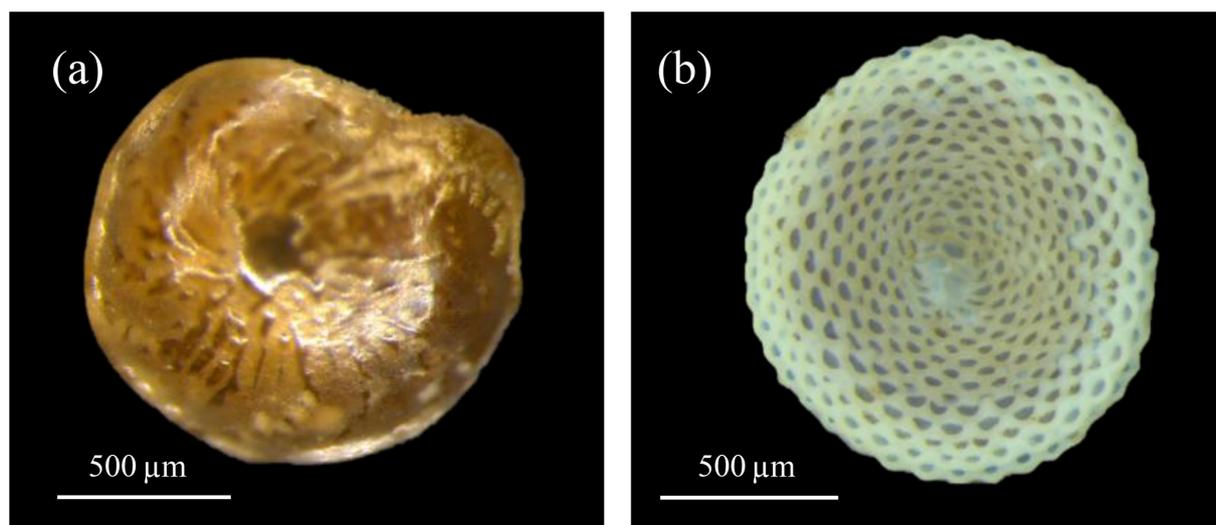
### 2.2 Laboratory manipulative experiments

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 tempera-

tures. These are both very accurate quantitative parameters. As such, they were chosen for this study, which 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  $\pm 0.5^\circ\text{C}$ ; temperatures were monitored using HOBO data loggers that recorded the temperature every hour. During the cultivating period, the samples were kept under a daily cycle of 12 h light–12 h darkness using a fluorescent light of  $\sim 30 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ . 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., 2016a, b, 2018; Titelboim et al., 2019). These light levels should not cause stress, since LBF acclimate rapidly to different light levels (in under 48 h, Ziegler and Uthicke, 2011), and thus the 10 d 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 10 d. Acclimation temperatures were optimal for each species:  $27^\circ\text{C}$  for *S. orbiculus* and  $25^\circ\text{C}$  for *A. lobifera*, and other conditions are as described in Sect. 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  $25^\circ\text{C}$  treatment and one *S. orbiculus* replicate from  $30^\circ\text{C}$ ) did not exhibit similar values of net oxygen production to 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 ( $1^\circ\text{C h}^{-1}$ ). The examined treatments ( $25, 30, 32, 35^\circ\text{C}$ ) 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 the low performance of the symbionts (*A. lobifera*  $25^\circ\text{C}$  and *S. orbiculus*  $30^\circ\text{C}$ ). After acclimation, following each week, the water was replaced with fresh natural seawater with a verified pH of 8.0–8.1 and salinity of 38.4–39.2. The replaced water from all the samples was transferred to airtight syringes, and then all oxygen samples were immediately measured. Alkalinity measurements were conducted over the next 2 d. To ensure that no changes occurred in this time frame, standard material was measured before and after the first and last sample of the set, respectively. Calcification rates ( $\mu\text{mol CaCO}_3$  per week per specimen) 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  $\text{CaCO}_3$ . These are determined by comparison to a control sample containing no foraminifera. Accuracy was assessed by analyses of the Scripps Institute of Oceanogra-



**Figure 1.** The holobionts examined in this study. (a) *Amphistegina lobifera* and (b) *Sorites orbiculus*. Note the greenish-brown colour of the symbiont algae.

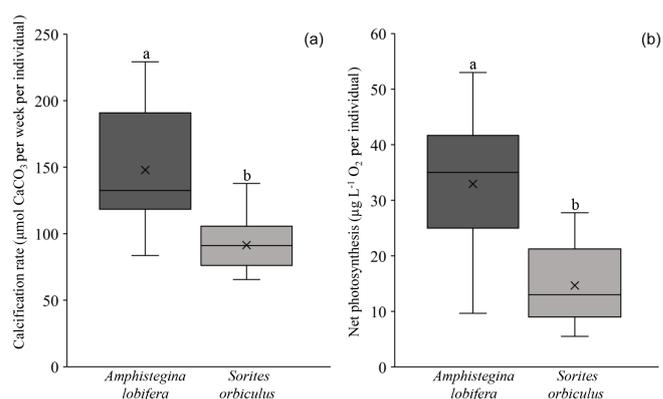
phy reference seawater (Batch 180) and an internal standard. The calcification rate involves high energetic consumption and as such is drastically influenced by stress levels of a calcifying organism and was especially shown to be related to thermal stress in benthic foraminifera (Evans et al., 2015; Schmidt et al., 2016b; Titelboim et al., 2019). Net photosynthesis ( $\Delta\text{O}_2 \mu\text{g L}^{-1}$  per specimen) was measured as net oxygen production compared with a control sample containing no foraminifera. Dissolved oxygen was measured using a 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, and 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 Kruskal–Wallis test was applied. Each was followed by the proper post hoc test. All statistical analyses are summarized in Table 1.

## 3 Results

Our experimental design takes biological variability in calcification rates and net photosynthesis between different



**Figure 2.** Activity baseline of the foraminiferal calcification rate (a) and symbionts' net photosynthesis (b) 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 holobionts. Error bars represent minimum and maximum values.

species into consideration. 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 baselines of both calcification and net photosynthesis are significantly different between *A. lobifera* and *S. orbiculus* (one-way ANOVA:  $p$  value  $< 0.001$ ; Fig. 2; Tables S1 and S2 in the Supplement). Hence, the thermal tolerance of the two holobionts was separately evaluated for each experiment.

**Table 1.** Description of all statistical analyses conducted in this study, including the statistical test performed, if data were transformed, and the number of samples in each data set.

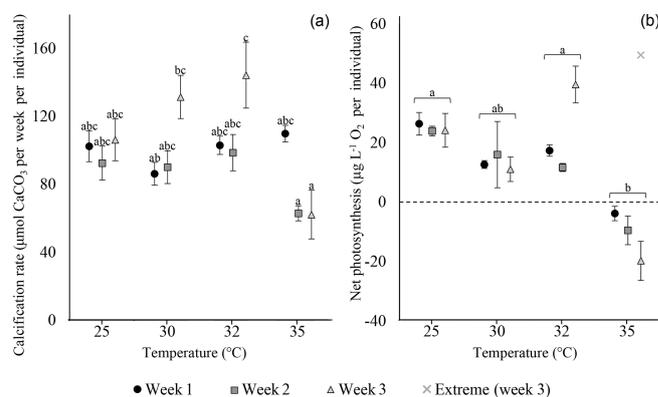
	Data	Description	Number of samples	Statistical analysis
Baseline	Calcification rate	Comparison between <i>S. orbiculus</i> and <i>A. lobifera</i> after acclimation period	<i>S. orbiculus</i> : 15 <i>A. lobifera</i> : 14	One-way ANOVA on log-transformed data
	Net photosynthesis	Comparison between <i>S. orbiculus</i> and <i>A. lobifera</i> after acclimation period	<i>S. orbiculus</i> : 15 <i>A. lobifera</i> : 15	One-way ANOVA on log-transformed data
<i>Sorites orbiculus</i>	Calcification rate	Comparison between four temperatures and 3 weeks	15	Two-way ANOVA and Tukey HSD test
	Net photosynthesis	Comparison between four temperatures	Weeks 1 and 2: 15 Week 3: 14	Kruskal–Wallis test and multiple comparisons
		Comparison between the 3 weeks		One-way ANOVA
<i>Amphistegina lobifera</i>	Calcification rate	Comparison between four temperatures and 2 weeks	15	Two-way ANOVA on log-transformed data and Tukey HSD test.
	Net photosynthesis	Comparison between four temperatures and 2 weeks	15	Two-way ANOVA and Tukey HSD test
	Third week bleaching of <i>A. lobifera</i>	Comparing the number of bleached specimens between four temperatures	15	One-way ANOVA on log-transformed data and Tukey HSD test.

### 3.1 *Sorites orbiculus* (porcellaneous-dinoflagellate holobiont system)

Calcification rates of *S. orbiculus* under the different temperature treatments exhibited the highest values at 25, 30, and 32 °C. A small decrease was observed at 35 °C, but this was not significant during the first week (Fig. 3; Tables S3.1 and S3.2). Net photosynthesis shows positive values under 25, 30, and 32 °C. At 35 °C, net photosynthesis was negative (Fig. 3; for statistical analyses, see Tables S4.1 and 4.2). Unaccountably, in week 3, one sample exhibited an abnormally high value (i.e. extreme in Fig. 3) with respect to previous weeks as well as to other replicates and thus was neither included in the average and error calculations nor in the statistical analysis.

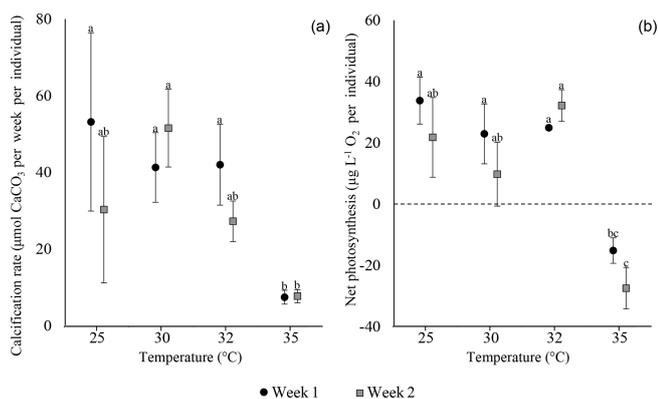
### 3.2 *Amphistegina lobifera* (hyaline diatom holobiont system)

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 25, 30, and 32 °C. At 35 °C calcification and net photosynthesis were both severely reduced and net photosynthesis was negative (Fig. 4; Tables S5 and S6). Between the second and third weeks, many specimens exhibit massive bleaching that occurred in different treatments be-



**Figure 3.** Calcification rates (a) and net photosynthesis (b) of *S. orbiculus*. Letters represent the results of the post hoc tests (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.

tween 25 and 32 °C in similar proportions (Tukey HSD post hoc test:  $p$  values  $> 0.1$ ; Table S7.2) and thus was clearly not related to the different temperature. Bleaching in the 35 °C treatment did not exceed two specimens per replicate (Table S11). For this reason, measurements of the third week are excluded from the results.



**Figure 4.** Calcification rates (a) and net photosynthesis (b) of *A. lobifera*. Note the synchronous negative response at 35 °C. Error bars are SE, and letters represent the results of the post hoc tests between temperatures and weeks (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, Symbiodiniaceae (Merkado et al., 2013; Pawlowski et al., 2001; Pochon et al., 2007). Both species are considered to be prominent calcifiers based on their massive occurrences and widespread distribution (Langer and Hottinger, 2000), and both record a gradual decline in physiological performance between 32 and 35 °C (Figs. 3–4). Both holobionts show thermal resilience up to 32 °C and a negative response at 35 °C (Figs. 3 and 4). However, they differ with 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 35 °C, whereas in *S. orbiculus* calcification is less dramatically reduced at 35 °C, indicating that it is more resilient to extreme SST than *A. lobifera*. Moreover, the Symbiodiniaceae symbionts exhibit stress earlier (already after the first week) than calcification, which 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* implies 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; Stühr et al., 2017; Schmidt et al., 2016; Hallock et al., 2006b). Hallock et al. (2006b) 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 that (1) the symbiont and host have similar thermal sensitivity 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 coral’s ability to cope with elevated temperatures is related to its 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 its symbiosis is limited to dinoflagellate from the Symbiodiniaceae “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, larger diversity of algal symbionts was associated with the more tolerant species (Stühr et al., 2018).

#### 5 Conclusions

Considering the role of LBF in the carbon cycle and as ecosystem engineers, their future with expected warming is a major concern. Previous studies 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 in 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 paper are available in the Supplement.

*Supplement.* The supplement related to this article is available online at: <https://doi.org/10.5194/bg-17-2341-2020-supplement>.

*Author contributions.* The study was designed by DT and DP. Sampling and culturing experiments were carried out by DP and DT using facilities provided by SA. Interpretation of data and writing of the paper were done by all authors: DP, DT, and SA.

*Competing interests.* The authors declare that they have no conflict of interest.

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