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Changing light levels induce photo-oxidative stress and alterations in shell density of *Amphistegina lobifera* (Foraminifera)

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ABSTRACT: Light is an important factor limiting the distribution of holobiont (host-algae) organisms in marine environments. Reef-dwelling large benthic foraminifera (LBF) are particularly sensitive to changes in light exposure, but their cryptic behaviour allows them to colonise different habitats across a light gradient. Yet, how foraminifera of the same species that live in different habitats respond to variations in light conditions, and the physiological and morphological attributes that govern that response, remain unclear. Here, we investigate how *Amphistegina lobifera*, a common and abundant LBF species in reef environments, collected from different reef sites across a light gradient, respond to changing light levels under lab-controlled conditions. Reduced light below their optimum level for growth and calcification caused a significant reduction in antioxidant capacity, and a significant decline in the density of newly added chambers. In contrast, elevated light caused significant reduction in survivorship, induced bleaching and photo-oxidative stress, and caused thickening of external chambers. Reef site did not have a significant effect on most of the responses, and populations collected from different reef sites showed similar sensitivity to changes in irradiance below and above their optimum level. The capacity to regulate symbiont density and to host a diversity of symbiotic diatoms within *Amphistegina* could be a reason for their ability to acclimate rapidly to different light levels. However, prolonged exposure to reduced light may result in a decrease in shell density and, as a consequence, a decline in carbonate production in reef environments.

KEY WORDS: Acclimation · Irradiance · CT scan · Antioxidant capacity · Foraminifera · Coral reef

INTRODUCTION

Large benthic foraminifera (LBF) are single-celled protists that harbour algae as photosymbionts and are restricted to a narrow set of environmental conditions, such as high light intensity and relatively clear, nutrient-poor waters of tropical and warm-temperate seas (Hallock 1999, Hohenegger 2004, Lee 2006). LBF play a crucial role in carbonate cycling in coral reef environments and contribute up to 80% of the global foraminiferal reef carbonate production (Langer 2008). In addition to their ecological importance in carbonate production in reef systems, LBF have been widely used as indicator organisms to detect changes in water quality in coral reef waters (Uthicke & Nobes 2008, Oliveira-Silva et al. 2012, Prazeres et al. 2012). This is due to their dependence on algal symbionts for both growth and calcification, and the relatively short life spans of LBF compared to long-lived reef corals (Hallock et al. 2003, 2006a).

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Owing to recent coral bleaching events and reef degradation both from climate change and increased local stressors on reefs (Pandolfi et al. 2011), understanding the maintenance and breakdown of this symbiosis has become of great interest (Lee 2006).

Amphistegina is the most common and abundant LBF genus found in coral reefs worldwide (Hallock 1999) and hosts diatoms as endosymbionts (Lee 2006). Members of this genus utilise cryptic behaviour to avoid damage from intense light, which allows survival in shallow water (Zmiri et al. 1974, Hallock 2000, Fujita 2004). They are mainly found attached to phytal substrata (Muller 1974) and reef rubble in the benthos, especially in areas that are somewhat shaded (Fujita 2004). The optimum light intensity for growth in the laboratory varies by species, and is about 1 to 2% of tropical sea-surface light intensity for *A. lessonii* and *A. gibbosa* (Hallock et al. 1986, Williams & Hallock 2004). *Amphistegina* spp. have been observed to exhibit symbiont loss (i.e. bleaching) in both western Atlantic and Indo-Pacific species (Hallock & Talge 1994, Hallock 2000). Moreover, experimental studies have documented symbiont loss in response to photic or thermal stress in *A. gibbosa* (Talge & Hallock 2003), *A. radiata* (Schmidt et al. 2011) and *A. lobifera* (Prazeres et al. 2016). A key difference between bleaching in corals and foraminifera is that events of mass bleaching in corals have correlated most consistently with elevated sea-surface temperatures, while bleaching in *Amphistegina* is mainly associated with photo-inhibitory stress (Talge & Hallock 2003, Hallock et al. 2006a), but can also be triggered by increased water temperature (Talge & Hallock 2003, Schmidt et al. 2011).

The quality and quantity of solar radiation reaching the seafloor in reef and coastal waters is a critical environmental parameter that has been modified by human activities (Hallock et al. 2006b). For example, inshore reefs of the Great Barrier Reef (GBR) are subject to high levels of terrestrial runoff, which has increased in intensity as a consequence of the expansion of agricultural practices in northern Queensland catchments following European settlement in the 1850s (Brodie et al. 2011). As a result, more sediment reaches the inshore reefs, and light levels tend to be lower than at their mid- and outer-shelf counterparts. As turbid waters are usually rich in dissolved organic matter, these particles tend to attenuate the high-energy, short-wavelength radiation (blue to UV), which is primarily responsible for bleaching in LBF (Hallock et al. 2006b). Although light is an important environmental factor influencing the physiology and distribution of tropical benthic foraminifera (Hohenegger et al. 1999), Nobes et al. (2008) demonstrated that light is not a limiting factor affecting the distribution of LBF in shallow habitats (between 2 and 13 m depth) of the GBR. Even though LBF, such as *Amphistegina* spp., are abundant throughout the GBR, their capacity to tolerate and regulate biochemical functions in response to changing light levels could vary according to their local habitat, as demonstrated for changes in temperature and nitrate levels (Prazeres et al. 2016).

The overall aim of this research was to compare and determine the physiological and morphological effects of changing light levels in the LBF *A. lobifera* collected from different regions of the GBR by exposing them to different light levels in laboratory-controlled conditions. *A. lobifera* is the shallowest-dwelling species of the genus, well adapted to high light intensity (up to 100% surface irradiance) (Hohenegger et al. 1999). Köhler-Rink & Kühl (2000) reported irradiance at maximum photosynthetic rate as 95 µE m^{-2} s^{-1}. This species characteristically produces thicker shells than other, deeper-ranging species (Hallock & Hansen 1978), as growth rates and shell thickness are mainly dictated by water motion and light levels (Ter Kuile & Erez 1984), both of which decrease exponentially with depth. *A. lobifera* exposed to low light levels will generally produce smaller, thinner shells than those individuals exposed to high irradiance (Hallock & Hansen 1978, Hohenegger 2009). We analysed survivorship, bleaching frequency and antioxidant capacity. To investigate the effects of different light levels in the calcification process, we also calculated growth rates and density of the shell chambers. We hypothesized that *A. lobifera* individuals living in environments with stable light levels would be more sensitive to changes in irradiance than those individuals that experience greater variations in sunlight exposure.

MATERIALS AND METHODS

Sample collection and experimental approach

Live adults individuals of *Amphistegina lobifera* were recovered from dead coral rubble collected from different sites across a light gradient on the northern section of the GBR, using a long-established sampling procedure (Hallock et al. 1986). Samples were collected by SCUBA divers from the leeward slope of reefs located on (1) the inner-shelf — Martin (14° 45.3′ S, 145° 20.1′ E) and Linnet (14° 46.7′ S, 145° 20.3′ E) reefs, (2) the mid-shelf — Lizard Island

(14° 14.4′ S, 145° 27.9′ E) and (3) the outer-shelf — Yonge (14° 35.8′ S, 145° 37.4′ E) and Day (14° 29.5′ S, 145° 30.9′ E) reefs between 6 and 9 August 2013. All samples were collected at depths of 6.0 to 9.5 m (corrected to lowest astronomical tide). Samples were amalgamated into the following 3 groups: inner-, mid- and outer-shelf individuals. Rubble was brought to the laboratory located at the Lizard Island Research Station and processed following methods described by Hallock et al. (2006a). Briefly, pieces of rubble were scrubbed using a toothbrush and resulting sediments were poured into Petri dishes for further separation of *A. lobifera* specimens. Only adults (>0.7 mm in diameter) with a uniform brown colour displaying pseudopodial activity (indicative of health) were selected for the experiment.

The effect of different light levels on the foraminifera was studied over a period of 30 d in flow-through (500 ml min^{-1}) outdoor aquaria at the Lizard Island Research Station. A total of 3 light treatments were used consisting of 5 replicates (5 l tanks) each. A total of 80 specimens from each reef site group were haphazardly assigned and placed into glass Petri dishes (10 cm in diameter) in each replicate tank. Each Petri dish contained pieces of dead coral skeleton, so that individuals could attach themselves. Each tank housed 3 Petri dishes from each reef site group, which were used as follows: (1) in the first Petri dish, 40 specimens from each group were haphazardly assigned for bleaching frequency and survivorship assessment; (2) the second Petri dish contained 20 specimens for the total antioxidant capacity (TAC) assay; and (3) for each replicate tank, a group of 5 randomly selected individuals was placed in a third Petri dish to determine growth rates (using surface area) and study of internal structures. In total, 65 specimens per reef site group (i.e. inner-, mid- and outer-shelf) per replicate tank were used in this study.

Light intensity varies between inshore and offshore reefs. Measured *in situ* light levels on the inner-shelf reefs received an average of 15 to 25 µE m^{-2} s^{-1} at midday at 6 m depth, whereas the mid- and outer-shelf study reefs regularly experienced light levels above 150 µE at the same depth. As we used natural sunlight in the experiments, light levels varied according to the time of day. For our irradiance treatments, light levels were reduced to 330.2 ± 10.2, 80.4 ± 8.5 and 25.2 ± 5.2 µE m^{-2} s^{-1} at midday using shade cloth. Light levels were constantly monitored throughout the duration of the experiment using HOBO light loggers. Temperature was kept constant at 24 ± 0.6°C and nutrient levels were 0.46 ± 0.03 and 0.02 ± 0.006 µM of dissolved inorganic nitrate and phosphate, respectively, throughout the duration of the 30 d experiment.

Survivorship and bleaching frequency

For assessment of survivorship and bleaching frequency, we started each experiment with 40 randomly assigned individuals per replicate tank. To determine the frequency of bleaching we counted the number of individuals that showed any sign of symbiont loss (ranging from small white spots to extensive white or 'mottled' areas) and determined the percentage of bleached individuals following Hallock et al. (2006a). Likewise, the number of dead individuals (empty shells) was assessed after 30 d, and survivorship was calculated as percentages. Individuals that underwent asexual reproduction, as evidenced by empty shell surrounded by newborn offspring, were not included in the total number of dead individuals.

Growth rate

Surface area was analysed by digital photographs taken of the cross-sectional shell surface area throughout the experiment (Uthicke & Altenrath 2010, Prazeres et al. 2015). Digital photographs (Microscope SteREO Discovery V8 with attached AxioCam Arc 5s, Zeiss) of each of the 5 assigned individuals per tank were taken at the beginning and at the end of the experiment. Photographs were analysed using the software ImageJ (Rasband 1997) to automatically trace the surface area of the specimens and calculate area gain (derived from pixel area gain) over the course of the experiment. Growth rates of *A. lobifera* were based on overall means of each Petri dish, as the shells of this species do not possess characteristic differences that would allow the tracking of individual specimens. Growth rate (percentage surface area per day) was determined using the equations of Ter Kuile & Erez (1984) as follows:

$$\lambda = \ln (S_f/S_i) \times 1/t$$
$$R = 100 \times \lambda$$

where λ is the growth constant, S_f and S_i are the final and initial surface area size, respectively, t is the time in days and R is the growth rate in percentage. Average initial surface area of analysed specimens did not deviate between reef sites for each treatment (partly nested ANOVA: light level × reef site(tank), $F_{4,36}$ = 1.85, p = 0.14; Levene's test: p = 0.27).

Determination of TAC

The TAC assay was performed to measure the biological resistance to various kinds of oxyradicals in order to predict their adverse effects on the physiological condition of the holobionts (Amado et al. 2009). TAC was measured using the fluorescence technique following the protocol described in Amado et al. (2009) and modified by Prazeres et al. (2012). Each sample containing 20 specimens was homogenized in a Tris-HCl (100 mM) buffer containing EDTA (2 mM) and $MgCl_2$ (5 mM). Additional samples from each site were used to calculate TAC prior to beginning the experiment. Protein concentration was adjusted to 0.75 mg protein ml^{-1} using the Quant-iT Protein Assay (Life Technologies). We added 10 µl of supernatant from each sample to a black 96-well microplate together with 127.5 µl of reaction buffer. For the reaction, 7.5 µl of 2,2′-Azobis (2-methylpropionitrile) (1 mM) was further added to each sample. Finally, the fluorescent probe 2′,7′-dichlorofluoresceindiacetate (H_2DCF-DA) was added to all wells at a final concentration of 40 µM. Fluorescence was read (excitation: 488 nm; emission: 525 nm) every 5 min in a microplate reader (Synergy2, BioTek) for 45 min at 37°C. Results were expressed as the inverse of the relative area and calculated according to Amado et al. (2009).

Analysis of internal structures

X-ray images were used to visualise internal structures and calculate shell density of individuals collected from different reef sites exposed to varying light levels. Analysis was performed on SkyScan 1174 as described by Gorog et al. (2012) and modified by Prazeres et al. (2015). Briefly, 5 specimens previously selected for the growth rate measurements were used in this analysis. Individuals were cleaned using a fine brush, washed in de-ionized water and oven-dried at 37°C. They were then placed inside the specimen chamber attached to the object manipulator using double-side adhesive tape. All individuals were visualised using 7.0 µm magnification and scanned in a 180° rotation using a 0.5 mm Al filter at 50 kV and 800 µA. Subsequently, cross-section images were reconstructed using the software SkyScan NRecon, and visualised using the software DataViewer. Shell chambers were grouped in internal (proloculus and oldest chambers), intermediate and external regions, and density was calculated using the software CT Analyser. Foraminiferal shell density values were calculated against water as standard.

Data analyses

This experiment was analysed as a blocked design (i.e. partly nested ANOVA), where 'tank' was the blocking random factor, and 'reef site' and 'light level' were within and between fixed factors, respectively. Bleaching frequency and survivorship were analysed with a generalised linear mixed model (GLMM) using the package 'lme4' in R (R Core Team 2013) as they represent percentages (Warton & Hui 2011). The GLMM was used to determine differences between reef sites and light levels. Significance of single and interaction effects of fixed factors was quantified using Type III SS for ANOVA. A factor detailing each reef site × light level combination was employed with a Tukey's HSD test, using the package 'multcomp'. Growth rate and final TAC were analysed using partly nested ANOVA as described above, with Type III SS. The initial antioxidant capacity (pre-exposure) was analysed using 1-way ANOVA to test for differences between reef sites. Growth rates data were logit transformed prior to the ANOVA (Warton & Hui 2011). In the case of shell density, we used a slightly different partly nested ANOVA design, which included 'chamber' as a fixed factor within 'reef site' and 'tank' factors. 'Chamber' refers to the position of the chambers within each individual shell (i.e. internal, intermediate and external). When indicated, ANOVA was followed by Tukey's HSD test. All data were checked for homogeneity of variance as well as normality prior to ANOVA analysis using Levene's and Shapiro-Wilks tests, respectively. Data shown as mean ± SEM, unless otherwise stated. Partly nested ANOVAs were conducted using the package 'stats' in R (R Core Team 2013). In all cases, the significance level adopted was 95% (α = 0.05). R scripts for the analyses can be found in the Supplement at www.int-res.com/articles/suppl/m549p069_supp.pdf.

RESULTS

Bleaching frequency did not vary between reef sites, but was significantly different between light levels, and the interaction between these 2 factors was also marginally significant (Table 1). Bleaching frequency was higher in individuals exposed to intermediate and high light levels than to low light (~40% versus <20%; see Fig. 1a). Bleaching frequency reached mean values of 51.0 ± 5.2% (mean ± SEM) in inner-shelf populations at the highest light levels (Fig. 1a).

Survivorship varied significantly among reef sites and at different light levels. Interaction between reef

Table 1. GLMM results for bleaching frequency and survivorship of *Amphistegina lobifera* populations collected from inner-, mid- and outer-shelf reefs of the northern GBR area exposed to varying conditions of light levels. **Bold**: significantly different at $p < 0.05$

Response variable	Source	df	χ^2	p-value
Bleaching frequency	Reef site	2	4.39	0.11
	Light level	2	35.71	**<0.001**
	Reef site × light level	4	9.20	**0.05**
Survivorship	Reef site	2	13.75	**0.03**
	Light level	2	6.56	**<0.001**
	Reef site × light level	4	9.70	**0.04**

sites and light levels was also significant (Table 1). Survivorship was high for *Amphistegina lobifera* collected from inner-shelf reefs in all treatments, remaining above 80% for all light levels tested. However, mid- and outer-shelf foraminiferal survivorship was significantly lower at high light incidence compared to other treatments and reef sites (Fig. 1b, Table 1), reaching mean percentages of 32.1 ± 3.1% and 38.7 ± 4.3%, respectively, after 30 d. Asexual reproduction occurred in intermediate and high light levels, and accounted for less than 5% of individuals in any given tank.

Growth rates (as determined by increase in shell area) were not significantly affected by different light intensities among reef sites (Table 2), and the interaction between these factors was also not significant. However, it is worth noting that the rate of increase in area was slightly reduced under light levels of 25 and 330 µE m^{-2} s^{-1} in all 3 shelf populations (Fig. 1c).

Pre-exposure antioxidant capacity was similar between reef sites (Fig. 2a). Changes in light level had no significant effect on antioxidant capacity responses after 30 d, but was significantly different among reef sites. Additionally, antioxidant capacity varied over time for most reef sites and treatments. The interaction between these factors was significant (Table 3). Individuals of *A. lobifera* collected from inner-, mid- and outer-shelf reefs responded similarly to the 3 light levels tested (Fig. 2b), and low and high light levels induced a reduction in antioxidant capacity by ~55% across all 3 populations after 30 d. Intermediate light level (80 µE m^{-2} s^{-1}) had a significant effect on only the mid-shelf population (Fig. 2b).

Shell density in the newly added chambers was significantly affected by changes in light levels, but it was not significantly different between reef sites. However, the interaction between reef sites and light levels was significant (Table 4). Reduction or increase in light caused a significant change in shell density, mostly to the newly added chambers. External chambers were consistently less dense than internal and intermediate chambers (Fig. 3a), but the magnitude of external chamber density changed according to light levels. Elevated light

Table 2. Partly nested ANOVA for growth rates (expressed as surface area) of *Amphistegina lobifera* individuals collected from inner-, mid- and outer-shelf reef sites of the northern GBR area exposed to varying conditions of light levels. No values were significantly different at $p < 0.05$

Source	df	MS	*F*	p-value
Light level	2	0.003	0.112	0.895
Residuals	12	0.026		
Reef site	2	0.023	0.343	0.713
Reef site × light level	4	0.124	1.81	0.160
Residuals	24	0.069		

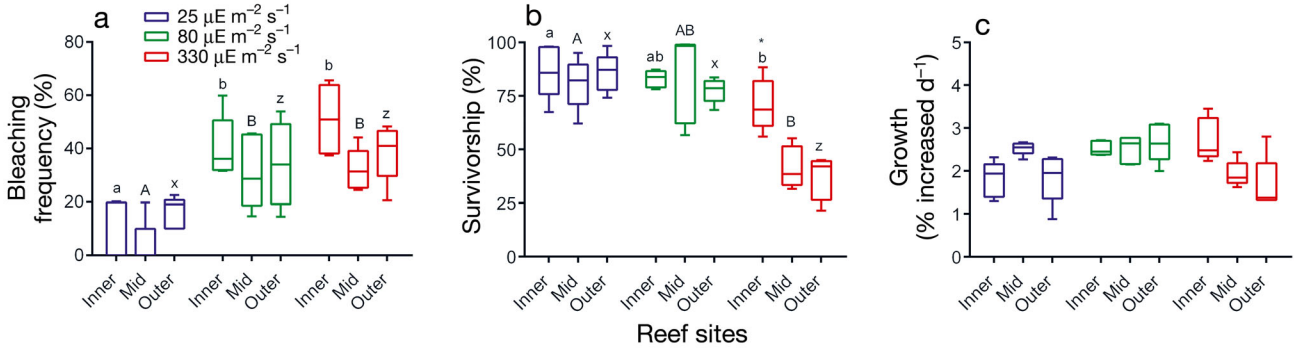

Fig. 1. (a) Bleaching frequency, (b) survivorship and (c) growth rates of *Amphistegina lobifera* collected from the inner-, mid- and outer-shelf reefs of the northern GBR. Individuals were exposed to different levels of light for a 30 d period. Data are plotted as mean ± 95% CI (boxes) (N = 5). Bars indicate minimum and maximum values. Different lowercase and uppercase letters indicate statistically significant differences (Tukey's HSD; $p < 0.05$) in mean values among experimental groups of individuals. Bars with no letters indicate no significant difference between treatments. *Significantly different mean values among sites within each experimental condition

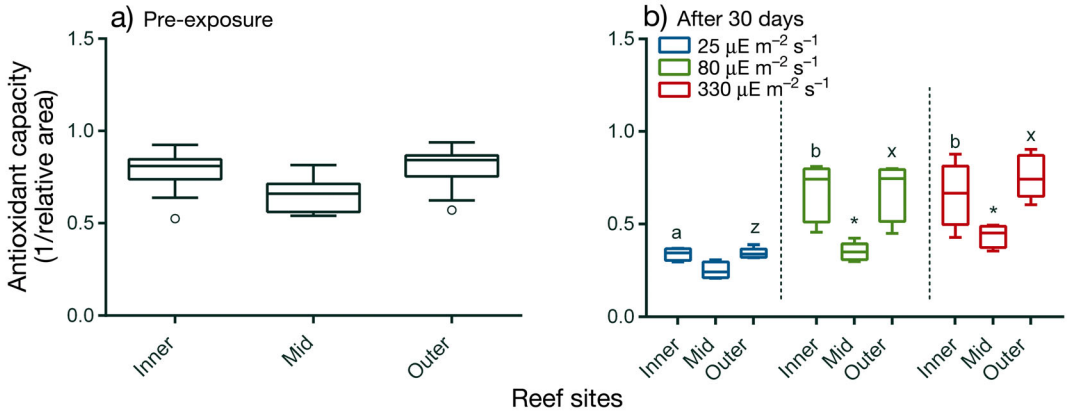

Fig. 2. Light effect on total antioxidant capacity of *Amphistegina lobifera* collected from the inner-, mid- and outer-shelf reef sites of the northern GBR. Antioxidant capacity was measured at (a) the beginning and (b) the end of the experiment. Data are plotted as mean ± 95% CI (boxes). (a) N = 15 per reef site and (b) N = 5 per light level × reef site combination. Bars indicate minimum and maximum values; circles indicate outliers. Different lowercase letters indicate statistically significant differences (Tukey's HSD; $p < 0.05$) in mean values among experimental groups, within the same reef site. Bars with no letters indicate no significant difference between treatments. *Significantly different mean values among reef sites within the same experimental group

levels induced higher densities of external chambers across all 3 populations, whereas reduced light levels resulted in less dense external chambers (Fig. 3b,c,d). Internal and intermediate chambers showed no change in trend across reef site and treatment.

DISCUSSION

Habitats of LBF are determined by a set of ecological gradients. The main factors influencing the distribution of symbiont-bearing LBF are temperature, light intensity, water movement and substrate

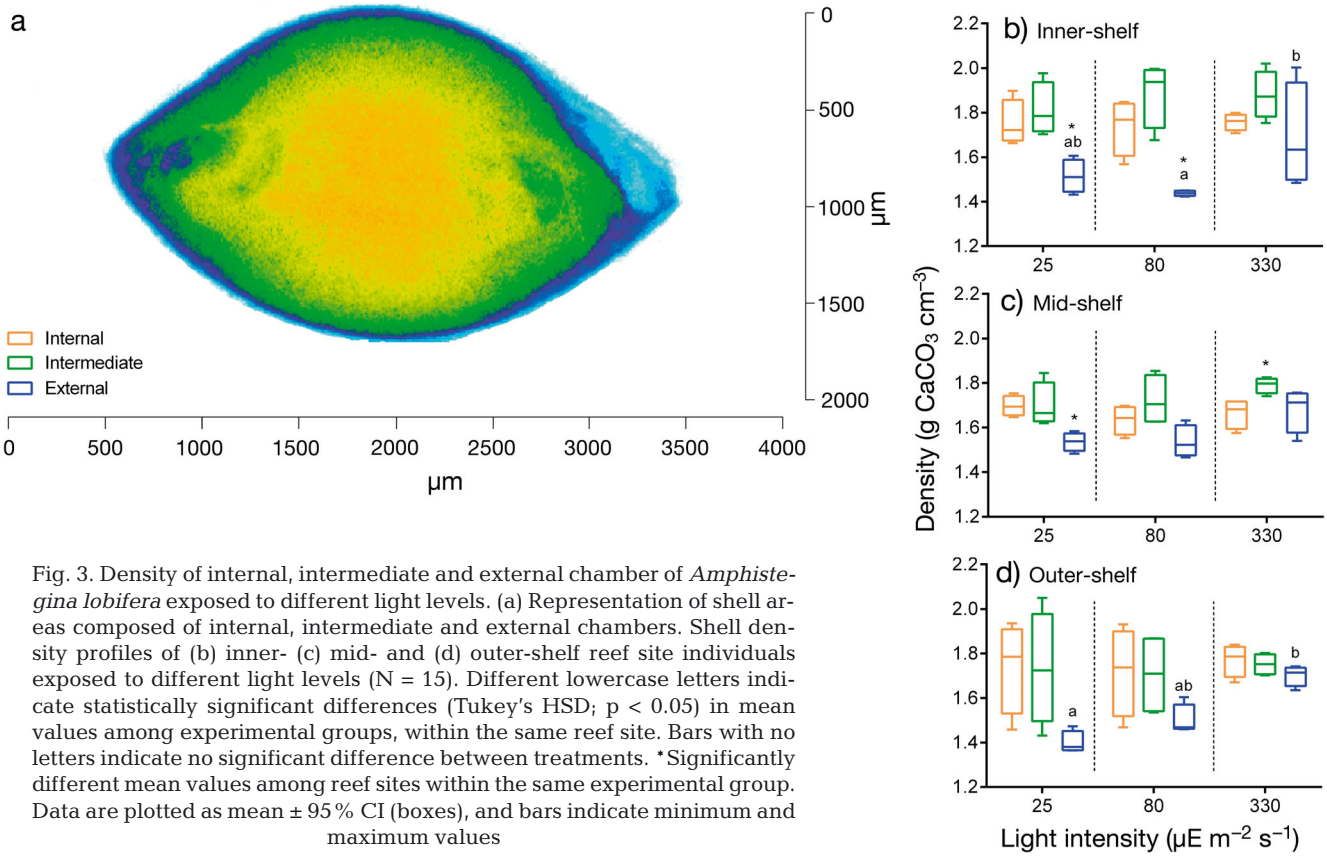

Fig. 3. Density of internal, intermediate and external chamber of *Amphistegina lobifera* exposed to different light levels. (a) Representation of shell areas composed of internal, intermediate and external chambers. Shell density profiles of (b) inner- (c) mid- and (d) outer-shelf reef site individuals exposed to different light levels (N = 15). Different lowercase letters indicate statistically significant differences (Tukey's HSD; $p < 0.05$) in mean values among experimental groups, within the same reef site. Bars with no letters indicate no significant difference between treatments. *Significantly different mean values among reef sites within the same experimental group. Data are plotted as mean ± 95% CI (boxes), and bars indicate minimum and maximum values

Table 3. Partly nested ANOVA results of antioxidant capacity assay of *Amphistegina lobifera* individuals collected from inner-, mid- and outer-shelf reef sites of the northern GBR area exposed to varying conditions of light levels after 30 d. All values were significantly different at $p < 0.05$

Source	df	MS	*F*	p-value
Light level	2	0.400	24.65	**<0.001**
Residuals	12	0.016		
Reef site	2	0.262	30.62	**<0.01**
Reef site × light level	4	0.029	3.362	**0.02**
Residuals	24	0.008		

Table 4. Partly nested ANOVA results of shell density of *Amphistegina lobifera* individuals collected from inner-, mid- and outer-shelf reef sites of the northern GBR area exposed to varying conditions of light levels. 'Chamber' refers to internal, intermediate and external chambers (see Fig. 3). All values were significantly different at $p < 0.05$

Source	df	MS	*F*	p-value
Light level	2	0.295	13.29	**<0.001**
Residuals	12	0.022		
Reef site	2	0.208	24.68	**<0.001**
Reef site × light level	4	0.061	7.28	**<0.001**
Residuals	24	0.008		
Chamber	2	0.604	93.79	**<0.001**
Chamber × light level	4	0.303	47.23	**<0.001**
Residuals	24	0.006		
Chamber × reef site	4	0.043	6.10	**<0.001**
Chamber × reef site × light level	8	0.025	3.52	**<0.01**
Residuals	48	0.008		

(Hohenegger et al. 1999). These foraminifera are phototaxic and move to areas where light conditions are optimal for their photosymbionts (Lee & Anderson 1991). Moreover, they can regulate symbiont densities in order to harness sufficient light energy to maintain the host–symbiont relationship (Lee 2006). Different species of LBF are adapted to different sets of environmental conditions, and some species are able to tolerate a broader range of fluctuation than others, especially temperature and light intensity (Hohenegger et al. 1999, Hohenegger 2004, 2009). Individuals of *Amphistegina lobifera* tend not only to move around the rubble but also to lift their tests from the substratum so that both sides of the test are exposed to light if required, and therefore maintain optimal photosynthetic levels (Köhler-Rink & Kühl 2000). The dose of radiant energy they actually experience in their microhabitat is unknown (Talge & Hallock 2003). Although foraminifera have the ability to modulate light levels in their microhabitats, in our study reef site and light levels had a significant effect on antioxidant capacity and changes in shell density of *A. lobifera*. Moreover, extended exposure to light levels above their optimum range yielded other effects, such as significant decreases in survivorship of mid- and outer-shelf populations.

Effects of local habitat

Our experiment showed that foraminifera from different reef sites had similar responses in bleaching frequency, and differed significantly in survivorship. Bleaching across all 3 populations exposed to high light levels was above 40%, and survivorship dropped to <50% in mid- and outer-shelf populations, even though light levels used here were still within the tolerance limits of *A. lobifera* tested in lab-controlled conditions using artificial light (Hallock 1981a). Inner-shelf populations showed similar results to those documented by Nobes et al. (2008), and survivorship of *Amphistegina* spp. ranged between 80 and 90% when exposed to similar natural light conditions (i.e. 375 µE m^{-2} s^{-1} at midday) after 30 d. Light spectra tested included both photosynthetic active radiation (PAR) and UV radiation, and ranged from 200 to 1200 nm (range detectable by the HOBO light meters used), as we used natural solar radiation in our study. Short wavelength radiation (<460 nm) induced symbiont loss in *A. gibbosa* (Talge & Hallock 2003, Williams & Hallock 2004). Symbionts of benthic foraminifera are able to adapt their pigment content to environmental light stress and probably possess protective mechanisms against intense radiation and UV damage (Köhler-Rink & Kühl 2001). Therefore the spectral quality of light received (PAR and UV), rather than intensity, appears to be a critical factor influencing bleaching and survivorship in *A. lobifera* (Talge & Hallock 2003, Williams & Hallock 2004), and possibly the genus.

Hallock (1981a) demonstrated that light levels of 2600 µW cm^{-2} (equivalent to 118 µE m^{-2} s^{-1}) boosted growth of *A. lobifera*, although the study used only a PAR light source. In our study, different light levels had no significant influence on growth rates of *A. lobifera* with respect to shell area, with inner-, mid- and outer-shelf foraminifera responding similarly to all light intensities tested. Growth did not vary after 30 d, and prior studies also revealed that growth rates in *Amphistegina* spp. (mainly *A. lobifera*) exposed to similar natural sunlight conditions did not differ significantly among treatments (Nobes et al. 2008), over the same length of time. Even though dif-

ferent light levels did not affect rates of increase in shell area, they had a significant effect on shell thickness, consistent with previous results by Ter Kuile & Erez (1984), across all 3 reef sites analysed. Additionally, *A. lobifera* individuals that live in shallow water develop thicker shells than those that occur at 45 m depth (Hallock & Hansen 1978). Our results demonstrate that light levels below an optimum range resulted in reduced shell density in external chambers (Fig. 3), which are the new chambers added by the foraminifera, and this trend was consistent among populations analysed. Foraminifera that are exposed to light levels above their optimum range calcify denser shells regardless of their collection site. This is likely to be a mechanism to protect the cell and symbionts from photo-oxidative stress, but still maintain photosynthesis levels (Hohenegger 2009). Moreover, the reduction in density between chambers formed prior to collection and those formed during the experiment is consistent with previous studies documenting the importance of water motion in shell thickness (Ter Kuile & Erez 1984, Hallock et al. 1986), as experimental treatments seldom provide the degree of water motion experienced in nature, especially at the relatively shallow depths (<10 m) from which our experimental specimens were collected.

Water transparency and light penetration are negatively influenced by terrestrial influx of inorganic and organic particles (Fabricius et al. 2013). *A. lobifera* from the inner-shelf reefs of the GBR mainly experience reduced light during seasonal flood events in the austral summer, when the influx of terrestrial material is high (Brodie et al. 2011, Devlin et al. 2012). Moreover, re-suspension of sediment by southeast winds at other times of the year can also increase turbidity in inshore reefs (Haynes 2001). Light intensities are higher during dry weather, when the quantity and quality of solar radiation that reaches the substrate changes (Hallock et al. 2006a). Mid- and outer-shelf reefs experience less turbid waters and, as a consequence, light intensity increases with greater distance from the mainland on the GBR (Fabricius et al. 2013). However, these habitats are not exposed to the same magnitude of fluctuation in water turbidity throughout the year, so even though the prevailing light intensity levels are higher, they are also more stable. Importantly, survivorship was significantly lower in mid- and outer-shelf populations than in those from the inner-shelf. *A. lobifera* from inner-shelf environments are less likely to experience high solar irradiance levels, but are exposed to wider variations in light levels than at offshore locations. As such, inner-shelf populations were more resistant to variation in light levels than mid- and outer-shelf individuals, thus confirming our initial hypothesis that individuals living in environments with stable light levels would be more sensitive to changes in irradiance than those individuals that experience greater variations in sunlight exposure.

Photo-oxidative stress and bleaching

The balance in the relationship between symbiont and host is very sensitive to any above-average increase in irradiance and temperature, and exposure of coral communities to temperatures as little as 1 to 2°C above the average summer maximum can lead to a breakdown of the symbiosis (Lesser 1997, Wooldridge 2014). Interestingly, light and temperature appear to yield a similar effect on bleaching and antioxidant capacity responses, as both factors are equally important for the endosymbiont photosynthetic system (Lesser et al. 1990). Both high and low light levels caused a decline in antioxidant capacity, possibly related to the symbiont density in the host. Oxidative stress has been suggested as one of the trigger mechanisms for bleaching in corals (Lesser 1997). Hallock et al. (2006a) argued that, as opposed to corals, *Amphistegina* bleach mainly in response to increases in short, high-energy wavelengths of solar radiation. Our findings also suggest that reduced antioxidant capacity can be induced by light above or below their optimum levels, and seems to be linked to bleaching responses in symbiont-bearing foraminifera. However, other factors such as temperature, as in corals (e.g. Lesser 1997), can also trigger bleaching in *A. lobifera* populations (e.g. Prazeres et al. 2016).

Oxidative stress is the production and accumulation of reduced oxygen intermediates such as superoxide radicals, singlet oxygen, hydrogen peroxide and hydroxyl radicals, and can potentially damage lipids, proteins and DNA (Lesser 2006). Owing to their photosynthetic nature, chloroplasts are naturally hyperoxic, produce reactive oxygen species (ROS) and are susceptible to oxidative stress. ROS in the chloroplast may damage Photosystem (PS) II, primarily through oxidative degradation of essential PS II proteins, and also inhibit the repair of damage to PS II (Lesser 2006). Elevated irradiance stress has been reported to cause degradation both of chloroplasts within the symbionts and the endoplasm of the host (Talge & Hallock 2003). Consistently, we observed that light levels above their optimal range induced oxidative stress and reduced holobiont antioxidant capacity (Fig. 2). Bleaching was detected across all

3 populations studied, but the low survivorship rate among mid- and outer-shelf reef populations demonstrates their higher sensitivity to intracellular production of ROS generated by diatom symbionts under high light levels when compared to inner-shelf counterparts. Loss of symbionts in some chambers (partial bleaching) might remediate further cellular damage in a short-term over-production of ROS by the diatoms, but bleaching cannot be considered a protective mechanism over prolonged periods of time, as Talge & Hallock (2003) found no evidence that bleached chambers could recover. Most shallow-dwelling LBF, including *A. lobifera,* rely on symbiosis for growth and calcification (Hallock & Hansen 1978, Hallock 1981a, Lee et al. 1991, Hohenegger 2009). As detailed studies of the effects of acute bleaching in *A. gibbosa* populations (Hallock et al. 2006a,b) demonstrated, effects such as increased shell damage, epiphytization and predation, reproductive failure and extreme decline in population densities, and bleaching in LBF and corals, clearly indicate susceptibility to environmental stresses for these holobiont assemblages.

In conclusion, it seems that changes in light levels affect *A. lobifera* similarly regardless of their collection site. Our results, and previous studies (Hallock & Hansen 1978, Hohenegger 1994), suggest that the direct effects of changes in light levels include changes in the antioxidant capacity and shell thickness of the holobiont, and have little influence on changes in growth rates and survivorship. The ability to regulate symbiont density within the cell, and to host a variety of symbiotic diatom species within *Amphistegina*, could be a reason for their capacity to acclimate rapidly to different environmental conditions, such as fluctuations in light levels (Lee 2006, Ziegler & Uthicke 2011). Regulation of antioxidant capacity and shell thickness confirms the ability of LBF to respond efficiently to changes in light levels. Nonetheless, prolonged exposure to light levels beyond their threshold of tolerance can still cause mortality of LBF. Changes in light intensity can also affect carbonate production in reef environments, as species that are able to calcify thicker and denser shells usually produced more calcium carbonate per individual than smaller, thinner individuals (Hallock & Hansen 1978, Ter Kuile & Erez 1984). LBF play a crucial role in reef sediment production and maintenance (Hallock 1981b, Langer 2008, Dawson & Smithers 2014), and decreases in light levels accompanied by increases in nutrients caused by terrestrial runoff, for example, can potentially reduce carbonate production in shallow areas of reef environments.

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