Original Article

Species variability in the response to elevated temperature of select corals in north-western Philippines

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Abstract

Thermal stress events threaten coral populations by disrupting symbiosis between the coral animal and microalgal symbionts in its tissues. These symbionts are key players in the response of the coral holobiont to elevated temperature. However, little is known about the microalgal symbiont type in select corals in the north-western Philippines and how they contribute to the differential responses of coral species. Based on sequencing of major ITS2 bands from DGGE, the dominant algal symbiont in *Acropora digitifera*, *A. millepora*, *A. tenuis* and *Favites colemani* was identified to be closely related to ITS2 type C3u, *Montipora digitata* contained ITS2 type C15, and *Seriatopora caliendrum* hosted ITS2 types similar to C3-Gulf and D1. Thin branching corals, such as *A. tenuis* and *S. caliendrum*, exhibited the greatest reduction in photochemical efficiency (Fv/Fm) and symbiont density at elevated temperature, followed by *M. digitata* and *A. millepora*, to a lesser extent. *A. digitifera* and *F. colemani* were least affected by the temperature treatment. Reduction in Fv/Fm and symbiont density was more apparent in *A. tenuis* and *A. millepora* than in *M. digitata* and *F. colemani*, although these species all host ITS2 type C3u symbionts. These results suggest that the impact of elevated temperature is influenced by factors apart from symbiont type. This highlights the importance of further studies on the diversity of corals and their microalgal symbionts in the region to gain insights into their potential resilience to recurring thermal stress events.

Introduction

Scleractinian corals are holobionts comprised of the coral host and associated bacteria, archaea, fungi, viruses and microalgal dinoflagellates known as *Symbiodinium* (Rohwer *et al.*, 2002). Corals build the framework of the reef, which supports a high biodiversity of species (Knowlton *et al.*, 2010). The ability of corals to build reef structures critically relies on their interaction with *Symbiodinium*, which provide much of their metabolic requirements (Muscatine & Porter, 1977). However, this symbiosis is sensitive to environmental perturbations, which can cause bleaching due to the degeneration or expulsion of symbionts from the coral host (Jokiel, 2004).

Many environmental triggers are known to induce coral bleaching, chief among which is rising seawater temperature associated with the changing global climate (Hoegh-Guldberg *et al.*, 2017; Hughes *et al.*, 2017). Increase in sea surface temperature (SST) may have contributed to the decline in coral cover within the Coral Triangle, a region in the Indo-Pacific with exceptional marine biodiversity (Carpenter *et al.*, 2008). This region is known to experience pronounced increases in SST during phases of the El Niño Southern Oscillation (ENSO) (McPhaden *et al.*, 2006), which can also trigger widespread coral bleaching.

Field and laboratory experiments confirm that corals exhibit different responses to thermal stress, with branching corals being more affected by bleaching events than massive corals (Marshall & Baird, 2000). These results were confirmed in a laboratory simulation experiment where branching corals, such as *Acropora* spp. and *Pocillopora damicornis*, exhibited greater susceptibility to bleaching at 32°C compared with massive corals, including *P. decussata*, *P. lutea* and *Montipora* sp. (Li *et al.*, 2008). Variation in the bleaching response among colonies within a species can also arise. For example, colonies of the massive coral *Porites* from the Palm Islands in the central Great Barrier Reef were more susceptible to bleaching than were *Porites* from Magnetic Island during a 1998 bleaching event (Marshall & Baird, 2000). One factor that might contribute to these variable responses is the association of the coral host with *Symbiodinium* of different thermal tolerances (Rowan, 2004). Indeed, corals inhabiting areas with highly variable temperature, nutrient and light conditions are dominated by thermally tolerant clade D *Symbiodinium* (Howells *et al.*, 2013; Keshavmurthy *et al.*, 2014; Silverstein *et al.*, 2015).

Despite advances in the study of coral and microalgal symbiont responses to elevated temperature, corals from regions within the Coral Triangle remain underexplored. To address this deficiency, we examined the symbiont associates and individual thermal stress responses of six scleractinian species from the reefs of Bolinao, north-western Philippines.

Materials and methods

Coral collection and acclimatization

Three colonies each of *Acropora digitifera*, *Acropora millepora*, *Acropora tenuis*, *Favites colemani*, *Montipora digitata* and *Seriatopora caliendrum* were collected in November 2016 from various sites within the Bolinao-Anda Reef Complex where each coral genus is abundant: *Acropora* (16′17.287N 120′00.448E; ∼5–9 m depth), *Favites* (16′18.623N 120′01.790E; ∼3–5 m depth), *Montipora* (16′26.513N 119′56.494E; ∼1–2 m depth) and *Seriatopora* (16′22.293N 120′00.228E; ∼4–6 m depth). Based on regular monitoring by the Bolinao Marine Laboratory, sea surface temperatures within the reef complex range from 25–32°C with annual mean temperature of 28.89 ± 0.90°C. Sample collection was conducted with the permission of the Philippines Department of Agriculture Bureau of Fisheries and Aquatic Resources (DA-BFAR Gratuitous Permit No. 0102-15). Colonies at least 10–15 m apart horizontally were collected to minimize genotypic similarity, although validation of genotypes was not conducted. Corals were fragmented into 2.5–5.0 cm long nubbins (∼70–80 fragments from each of 3 colonies per species) and pre-conditioned for 2 weeks in outdoor tanks with running seawater maintained at 28 ± 1°C and illumination under low photosynthetic photon flux density of ∼80–90 µmol m^{-2} s^{-1} on a 12:12 light-dark cycle. Fragments were tagged to keep track of their colony of origin. Healed fragments were then allowed to acclimatize for 2 weeks in indoor experimental tanks with running seawater maintained at 28 ± 1°C and illumination of ∼80 µmol m^{-2} s^{-1} on a 12:12 light-dark cycle.

Thermal stress experiments

Thermal stress experiments were conducted in 40 l tanks with constantly aerated, 10 µm-filtered flow-through seawater. Two independent replicate tanks were used for each temperature treatment. Seawater was pumped into each tank from chilled reservoirs maintained at 27°C and the temperature in individual experimental tanks was adjusted using submersible thermostat heaters. Flow rate was ∼5–8 l h^{-1} and additional mixing within the tanks was provided by 600 l h^{-1} pumps. All setups received illumination under low photosynthetic photon flux density of ∼80 µmol m^{-2} s^{-1} on a 12:12 light-dark cycle to avoid light stress. Light and temperature in each experimental tank was monitored using submersible loggers (Onset HOBO). Approximately 60–70 coral fragments (3–4 fragments from each of 3 colonies per species or a total of 10–12 fragments per species) were quickly transferred into each experimental tank set at either elevated temperature (32 ± 1°C, treatment) or ambient temperature (28 ± 1°C, control). Although this thermal shock treatment does not approximate what happens in nature, it allows investigation of the robustness of each species by emphasizing differences in acute temperature shock response, as has been done in other studies (Putnam *et al.*, 2010; Meyer *et al.*, 2011; Barshis *et al.*, 2013; Parkinson *et al.*, 2015; Hou *et al.*, 2018). Measurement of photochemical efficiency and collection of fragments for quantification of microalgal symbiont density were performed after 4, 24, 48 and 72 h of exposure.

Physiological measurements

Temperature stress in corals is readily detectable as a change in the photosynthetic fitness of associated algal symbionts measured using non-invasive pulse-amplitude modulated (PAM) fluorometry (Ralph *et al.*, 2015). The dark adapted photochemical efficiency (Fv/Fm) of coral fragments was measured using a diving PAM (Walz, Germany) at least 1 h after the onset of the dark phase of the light cycle. Two PAM measurements were taken from each of 5 coral fragments, taking care to include one to two randomly selected fragments from each of 3 colonies per species (N = 5 fragments per species per treatment). To quantify symbiont density, three coral fragments, one from each of 3 colonies per species (N = 3 fragments per species per treatment), were sacrificed at every sampling point and incubated for 1 h in 50 ml of 1 M NaOH to separate symbionts from the host tissues. The resulting cell suspension was spun at 4000 rpm for 15 min and the pellet washed twice with distilled water. Symbiont cells were resuspended in 30 ml distilled water before counting on a Neubauer hemocytometer (LeGresley & McDermott, 2010). Cell counts were taken four times for each coral fragment. The average cell count for each fragment was normalized to the total surface area of each fragment as determined using the wax dipping method (Veal *et al.*, 2010).

DNA extraction, PCR and sequencing

DNA was extracted from symbionts dissociated from host tissues using the Hot-Shot method (Truett *et al.*, 2000). PCR amplification of the internal transcribed spacer 2 (ITS2) region was performed using the primers ITSintFor2 and ITS2CLAMP (LaJeunesse & Trench, 2000). PCR reactions contained 1 µl of crude DNA template, 1× PCR buffer, 1.5 mM MgCl$_2$, 200 µM dNTP, 100 nM ITSintFor2, 300 nM ITS2CLAMP and 0.5 units of Taq DNA Polymerase (Invitrogen). PCR was carried out on a FlexCycler with a touchdown amplification protocol consisting of an initial denaturing step of 94°C for 3 min, 21 cycles of 94°C for 30 s, 62°C for 40 s and 72°C for 30 s with annealing temperature decreasing by 0.5°C at each cycle, followed by 15 cycles of 94°C for 30 s, 52°C for 40 s and 72°C for 30 s, and a final extension at 72°C for 10 min. PCR products were resolved on a 30–60% linear gradient of urea and formamide with 8% acrylamide (Sampayo *et al.*, 2009). Gels were run in 1× TAE at 60 V for 16 h at 60°C in a denaturing gradient gel electrophoresis (DGGE) apparatus (C.B.S. Scientific). After electrophoresis, the gel was stained with SYBR Gold nucleic acid stain in 1× TAE buffer, rinsed, and photographed. Distinct bands were excised from the gel and placed in 30 µl nuclease free water overnight to diffuse the DNA. The eluted DNA was re-amplified using the primers ITSintFor2 and ITSrev (LaJeunesse & Trench, 2000) for direct sequencing at First Base, Malaysia. Sequences were deposited in NCBI under accession numbers MG662689–MG662692.

Phylogenetic analysis

Microalgal symbiont ITS2 sequences obtained from DGGE were aligned to selected sequences from GeoSymbio (Franklin *et al.*, 2011) using MUSCLE (Edgar, 2004). Poorly aligned sequences were trimmed using Gblocks v0.91b (Talavera *et al.*, 2007). A total of 283 bp were retained after trimming. Phylogenetic analysis was conducted using maximum likelihood based on the Kimura 2-parameter model. Initial trees for the heuristic search were obtained automatically by applying neighbour-joining and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood (MCL) approach and then selecting the topology with superior log-likelihood value. Phylogenetic analysis was conducted using MEGA6 with 1000 bootstrap replicates (Tamura *et al.*, 2013).

Statistical analysis

Data were tested for homogeneity of variances using Levene's test and normality using the Kolmogorov–Smirnov test. Fv/Fm data did not pass normality tests, even after data transformations,

and were thus subjected to the non-parametric Friedman repeated measures test to analyse changes in Fv/Fm through time per temperature treatment for each species. Differences in Fv/Fm between temperature treatments were examined using a Mann–Whitney U test for each species. The Kruskal–Wallis test was also conducted to examine the differences in Fv/Fm among species for each temperature treatment. Differences in microalgal density between temperature treatments across time points were analysed using repeated measures ANOVA (analysis of variance) for each species. Separate ANOVA tests were conducted to clearly see the difference in microalgal density between temperature treatments for each species. Significant ANOVA results were further tested using a post-hoc Tukey's HSD test to compare pairwise differences in microalgal density between temperature treatments or between time points. A Bonferroni correction was applied on *P*-values to account for the increase in statistical error in multiple comparisons (Zar, 1984). To determine which species had a significant association between Fv/Fm and microalgal density, Spearman correlation tests were conducted. All tests were conducted in Statistica 6.

Results

Identification of microalgal symbiont ITS2 types

Sequencing of the major ITS2 DGGE bands from each coral species revealed that the dominant symbiont in the three acroporids and in *F. colemani* was most closely related to ITS2 type C3u (Figure 1). *Montipora digitata* hosted ITS2 type C15 while *S. caliendrum* hosted an ITS2 type similar to C3-Gulf (Hume *et al.*, 2015) and D1. Multiple lighter bands appearing above the prominent bands in DGGE are likely to be heteroduplexes or artefacts of PCR (LaJeunesse, 2002). No other phylotypes were identified using DGGE.

Effect of thermal stress on photochemical efficiency

Coral fragments maintained at the control temperature exhibited a dark-adapted photochemical efficiency (Fv/Fm) of 0.72 ± 0.02 (mean ± SD) for all species (Figure 2A). Sustained exposure of corals to elevated temperature resulted in a significant reduction in Fv/Fm over several days for some species (Table 1). *Acropora tenuis* showed the most immediate response, with Fv/Fm values dropping to 0.47 ± 0.04 after 24 h and then to 0.0 after 48 h, when fragments exhibited complete bleaching (Figure 2A). *Montipora digitata* also showed a significant decrease in Fv/Fm, reaching 0.52 ± 0.06 in 24–72 h. *Seriatopora caliendrum* revealed a gradual decrease in Fv/Fm values, dropping to 0.48 ± 0.02 after 48 h and then to 0.13 ± 0.18 after 72 h (Figure 2A). For *A. millepora*, a significant decrease in Fv/Fm to 0.49 ± 0.02 was observed only at 72 h. *Favites colemani* and *A. digitifera* showed a decrease in Fv/Fm at 48 h, but this was not significant relative to controls and appeared to recover by 72 h (Figure 2A).

Effect of thermal stress on microalgal symbiont density

After the first 4 h at 28°C, the microalgal symbiont density already varied significantly among the six scleractinian coral species (one-way ANOVA: F = 334.6005; *P* < 0.001; Figure 2B). *Acropora tenuis* exhibited the lowest symbiont density, which differed significantly from that of *F. colemani* and *S. caliendrum* (Tukey's HSD test: pairwise comparisons between species, *P* < 0.05), but not from *A. digitifera*, *A. millepora* and *M. digitata* (Tukey's HSD test: pairwise comparisons between species, *P* > 0.05).

Prolonged exposure to elevated temperature resulted in a concomitant decrease in symbiont density for some coral species
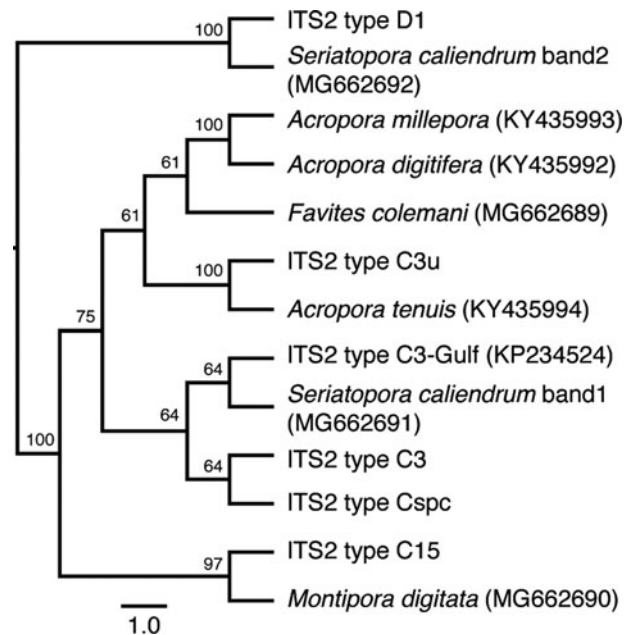

Fig. 1. Genetic identification of microalgal symbionts in colonies of six coral species at the Bolinao-Anda Reef Complex, Bolinao, Pangasinan, Philippines. Cladogram shows the sequence similarity of ITS2 bands from DGGE to known ITS2 types in GeoSymbio. NCBI accession numbers are shown in parentheses. Tree topology is based on maximum likelihood. Numbers on the branches indicate bootstrap values.

(Table 2). *Acropora tenuis* exhibited a significant reduction in symbiont density to a mean of 58% of that in the controls at 24 h (Tukey's HSD test: pairwise comparison between 28°C and 32°C, *P* < 0.05). This reduction in density was accompanied by visible bleaching, which progressed to a complete loss of symbionts by 48 h (Figure 2B). The symbiont density of *A. millepora* decreased to 57% of controls when exposed to elevated temperature, while *M. digitata* decreased to 34% of controls after 72 h, although both decreases were not significant (Figure 2B). *Seriatopora caliendrum*, on the other hand, retained a mean symbiont density of only 42% of controls after 4–48 h of elevated temperature exposure, which was significantly lower than that of the controls at all time points (Tukey's HSD test: pairwise comparisons between hours of exposure, *P* < 0.05; Figure 2B). *Seriatopora caliendrum* did not exhibit a sharp decline in symbionts until the 72 h timepoint, upon which bleaching was observed. In contrast, *F. colemani* and *A. digitifera* did not show a significant reduction in symbiont density from the control values, even after 72 h of exposure to elevated temperature (Tukey's HSD test: pairwise comparisons between hours of exposure, *P* > 0.05; Figure 2B).

Discussion

Our results indicate that coral species from various sites around the Bolinao-Anda Reef Complex, north-western Philippines, vary in terms of the dominant microalgal symbiont type present within their tissues. The three acroporids and *F. colemani* hosted ITS2 type C3u, a host generalist that has also been detected in other corals in the western and north-eastern Indian Ocean (LaJeunesse *et al.*, 2010). *Montipora digitata* hosted ITS2 type C15, which is the same as the symbiont identified in *M. digitata* from other sites (Fisher *et al.*, 2012; Krueger *et al.*, 2015). *Seriatopora caliendrum* hosted two thermotolerant ITS2 types, C3-Gulf and D1. ITS2 type C3-Gulf was first detected in Red Sea corals (Hume *et al.*, 2015), while D1 has been found in corals
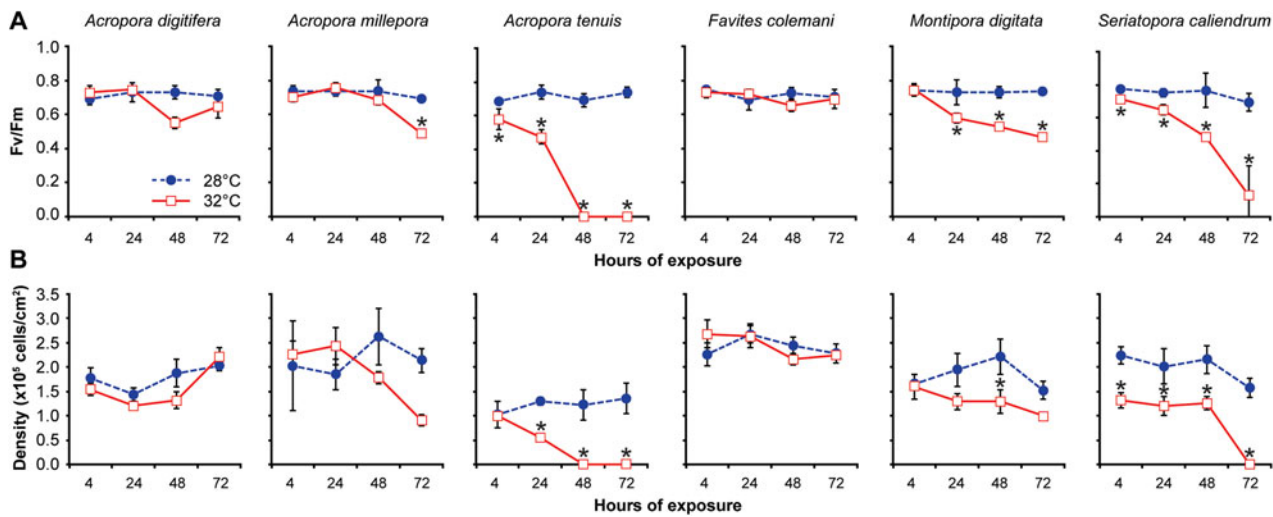
Fig. 2. Variation in the photochemical efficiency and density of microalgal symbionts among six coral species exposed to two treatments over 72 h. (A) Photochemical efficiency (Fv/Fm), and (B) Algal symbiont density (×10⁵ cells per cm² of coral surface area). Coral fragments of each species were subjected to a treatment temperature of 32°C (red-bordered squares) or a control temperature of 28°C (blue circles) for 4, 24, 48 and 72 h. Data are presented as means ± standard deviation of N = 3–5 coral fragments examined during each time period for each species. Asterisks indicate significant differences between treated (32°C) and control (28°C) values at each time point (Tukey's HSD test, $P < 0.05$).

Table 1. Summary results of non-parametric tests of variation in photochemical efficiency (Fv/Fm) between two temperature treatments (28 and 32°C) across four time points (4, 24, 48, 72 h) in each of six coral species

	28°C			32°C		
Species	df	χ^2	*P*-value	df	χ^2	*P*-value
(A) Friedman repeated measures ANOVA tests						
Acropora digitifera	3	2.52	ns	3	11.64	ns
Acropora millepora	3	2.52	ns	3	15	**0.0018**
Acropora tenuis	3	8.76	ns	3	15	**0.0018**
Favites colemani	3	5.4	ns	3	7.08	ns
Montipora digitata	3	0.6	ns	3	15	**0.0018**
Seriatopora caliendrum	3	7.8	ns	3	15	**0.0018**
Species	df	Z	*P*-value			
(B) Mann–Whitney *U* tests						
Acropora digitifera	1	1.36	ns			
Acropora millepora	1	2.61	**0.0090**			
Acropora tenuis	1	2.61	**0.0090**			
Favites colemani	1	0.10	ns			
Montipora digitata	1	2.61	**0.0090**			
Seriatopora caliendrum	1	2.61	**0.0090**			
Temperature	df	H	*P*-value			
(C) Kruskal–Wallis tests						
28°C	5	8.669	ns			
32°C	5	44.831	**<0.0001**			

(A) Friedman repeated measures ANOVA tests of changes across time points (4, 24, 48 and 72 h) in each of the two temperature treatments (28 and 32°C) for each species. (B). Mann–Whitney U tests of differences between the two temperature treatments for each species at the 72 h time point. (C) Kruskal–Wallis tests of differences among the six species in each of the temperature treatments.
P-values in bold are significant at the 0.05 level after Bonferroni correction.

that experience a high cumulative frequency of thermal stress anomalies (Stat & Gates, 2011). These findings suggest that the examined corals may select for specific types of symbionts (Yamashita *et al.*, 2014). It should be noted, however, that it is possible for corals to host other rare symbiont types that may

contribute to the overall holobiont response but may not have been detected by DGGE.

Interspecies differences in thermal stress response were observed. Most of the corals were able to withstand sustained exposure to high temperature for up to 72 h, except for the thin

Table 2. Summary results of repeated measures ANOVA of microalgal symbiont density between two temperature treatments (28 and 32°C) across four time points (4, 24, 48 and 72 h) for each of six coral species

Species	Factors	df	F	P
Acropora digitifera	Temperature	1	10.23	ns
	Time	3	19.14	**<0.001**
	Temperature × Time	3	3.89	ns
Acropora millepora	Temperature	1	1.39	ns
	Time	3	4.52	ns
	Temperature × Time	3	8.04	**<0.01**
Acropora tenuis	Temperature	1	66.89	ns
	Time	3	7.65	**<0.01**
	Temperature × Time	3	18.97	**<0.001**
Favites colemani	Temperature	1	0.04	ns
	Time	3	7.11	ns
	Temperature × Time	3	4.36	ns
Montipora digitata	Temperature	1	154.46	**<0.001**
	Time	3	3.62	ns
	Temperature × Time	3	2.62	ns
Seriatopora caliendrum	Temperature	1	70.77	**<0.01**
	Time	3	35.55	**<0.001**
	Temperature × Time	3	4.71	ns

P-values in bold are significant at the 0.05 level after Bonferroni correction.

branching corals, *A. tenuis* and *S. caliendrum*. These two corals showed the greatest reduction in photochemical efficiency and symbiont density, with bleaching after 48–72 h. *Montipora digitata* and *A. millepora* showed a less drastic reduction in photochemical efficiency and density, while *A. digitifera* and *F. colemani* were the least affected by sustained exposure to high temperature. Taking into consideration both photochemical efficiency and symbiont density changes over prolonged exposure to elevated temperature, the scleractinian corals of the Bolinao-Anda Reef Complex may be ranked in terms of decreasing thermal susceptibility in the following order: *A. tenuis* > *S. caliendrum* > *M. digitata* ≥ *A. millepora* > *A. digitifera* > *F. colemani*.

Interestingly, the temperature responses of the corals could not be directly correlated with the identity of their associated symbionts, nor with the morphology of the coral colony. The association of *M. digitata* with ITS2 type C15 was robust to heating, as has been reported by other studies (Fisher *et al.*, 2012; Krueger *et al.*, 2015). In contrast, the presence of two thermotolerant lineages in *S. caliendrum* did not translate to greater tolerance. This suggests that other factors may contribute to the overall response of the coral holobiont.

Amongst the corals hosting ITS2 type C3u, *A. tenuis* and *A. millepora* exhibited greater susceptibility to thermal stress compared with *A. digitifera* and *F. colemani*. In general, fast-growing branching corals are thought to be more susceptible to bleaching compared with slower-growing massive corals. This may be attributed to the accumulation of more harmful oxygen free radicals in organisms with higher metabolic rates (Lesser, 1997; Baird & Marshall, 2002). Variability in bleaching susceptibility has been reported in acroporid corals, which could be related to differences in the growth rate of the different branching morphologies (Dornelas *et al.*, 2017; Hoogenboom *et al.*, 2017).

The examined coral species with finer branches, such as *A. tenuis* and *S. caliendrum*, were most susceptible to thermal stress compared with the other branching corals, *A. millepora*, *M. digitata* and *A. digitifera*, or to the massive coral, *F. colemani*. These differences may be due to differences in host morphology, tissue thickness and light scattering properties (Enríquez *et al.*, 2005). In general, acroporids and other branching corals have thinner tissues compared with massive corals (Loya *et al.*, 2001). Thick tissue layers can expand and contract to provide a rapid and flexible means of regulating radiant flux and protecting symbionts via self-shading (Loya *et al.*, 2001; Coles & Brown, 2003). Thicker tissues may also translate to a higher density of microalgal symbionts, as observed for *F. colemani*, which may contribute to self-shading of symbiont cells and protection from bleaching. Studies on sea anemones and corallimorpharians further support the photoprotective role of thicker host tissues (Kuguru *et al.*, 2007; Dimond *et al.*, 2012).

The Bolinao-Anda Reef Complex in north-western Philippines faces the South China Sea and is an area that experiences high temperature variation (Peñaflor *et al.*, 2009). Thus, it is likely that the corals that thrive here have adapted to recurrent thermal stress in the area (Yu, 2012). Remarkably, *M. digitata*, which normally thrives on shallow reef flats that experience extreme temperature variation, exhibited greater tolerance to prolonged exposure to elevated temperature compared with the other corals with fine branching morphology. Its frequent exposure to high temperature and high light intensity in its shallow habitat may have contributed to this tolerance to sustained stress (Yap *et al.*, 2014). Other researchers have similarly demonstrated that corals grown at high temperature survive heat stress better than those grown at lower temperature (Middlebrook *et al.*, 2008; Howells *et al.*, 2013). Pre-conditioning or exposure of corals to slow warming rates also leads to overall differences in the severity of bleaching responses exhibited during thermal stress events (Brown *et al.*, 2002; Middlebrook *et al.*, 2008; Bellantuono *et al.*, 2011). This prior exposure may stimulate protective mechanisms, such as increased concentrations of certain pigments (Salih *et al.*, 2000; Dove *et al.*, 2008; Middlebrook *et al.*, 2008), UV-protective compounds (Yakovleva *et al.*, 2004; Baird *et al.*, 2009), enzymatic antioxidants (Richier *et al.*, 2005; Lesser, 2006), or expression of heat shock proteins (Rodriguez-Lanetty *et al.*, 2009; DeSalvo *et al.*, 2010; Traylor-Knowles *et al.*, 2017), which help reduce bleaching susceptibility.

Previous studies that used a short thermal shock regime to assay coral responses to thermal stress also reported variable temperature tolerance in different coral species. For example, *P. damicornis* and *S. hystrix* from Nanwan Bay in southern Taiwan did not exhibit significant changes in Fv/Fm after 12 h of exposure to thermal shock at 30, 28 and 21°C (Putnam *et al.*, 2010). On the other hand, *Galaxea fascicularis* from Luhuitou, Hainan, China, showed symbiont density reduction, bleaching and tissue lysis after just 10 h at 32°C (Hou *et al.*, 2018). A rapid increase in temperature from 29 to 34°C over 3 h also resulted in some bleaching in *A. nana* from Ofu Island in American Samoa (Bay & Palumbi, 2015). Clearly, the responses of corals to stress are affected by many factors, such as host species, type of symbiont, coral colony morphology and environment, among others. Recent studies are also beginning to uncover variations in physiological response that may be governed by genotypic

differences in corals hosting the same types of symbiont (Parkinson *et al.*, 2015).

The findings from these studies and others suggest that corals may have a multistage process for acclimation, with a rapid response mechanism to manage acute heat stress, which disrupts proteostasis and induces expression of protein folding chaperones and proteases (Oakley *et al.*, 2017), as well as a slower response that changes background gene expression and physiological function to anticipate future stress events (Bay & Palumbi, 2015). Thus, corals that regularly encounter thermal fluctuations in their natural habitat may already have higher baseline expression of transcripts encoding the proteins required for cellular repair and homeostasis (Barshis *et al.*, 2013), as well as gene regulatory mechanisms that allow the coral to mount a rapid cellular response in the event of acute thermal stress (Gajigan & Conaco, 2017). It would therefore be of interest to determine the gene expression dynamics that accompany the variable physiological responses of corals to environmental stress. Further investigations are also warranted to reveal how complementation between different coral host species and symbiont types contributes to the susceptibility or tolerance of the holobiont to environmental stressors.

In summary, we have shown differences in the response of different coral species to thermal stress through the use of photochemical efficiency and symbiont density as indicators of bleaching susceptibility. We also provide baseline information on the symbiont types present in common corals in this region. The findings highlight the role of the host in coral bleaching susceptibility and provide insights into how coral populations in the Coral Triangle region might respond to recurring thermal stress events.

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References

Baird AH and Marshall PA (2002) Mortality, growth and reproduction in scleractinian corals following bleaching on the Great Barrier Reef. *Marine Ecology Progress Series* 237, 133–141.

Baird AH, Bhagooli R, Ralph PJ and Takahashi S (2009) Coral bleaching: the role of the host. *Trends in Ecology and Evolution* 24, 16–20.

Barshis DJ, Ladner JT, Oliver TA, Seneca FO, Traylor-Knowles N and Palumbi SR (2013) Genomic basis for coral resilience to climate change. *Proceedings of the National Academy of Sciences USA* 110, 1387–1392.

Bay RA and Palumbi SR (2015) Rapid acclimation ability mediated by transcriptome changes in reef-building corals. *Genome Biology and Evolution* 7, 1602–1612.

Bellantuono AJ, Hoegh-Guldberg O and Rodriguez-Lanetty M (2011) Resistance to thermal stress in corals without changes in symbiont composition. *Proceedings of the Royal Society B: Biological Sciences* 279, 1100–1107.

Brown B, Dunne R, Goodson M and Douglas A (2002) Experience shapes the susceptibility of a reef coral to bleaching. *Coral Reefs* 21, 119–126.

Carpenter KE, Abrar M, Aeby G, Aronson RB, Banks S, Bruckner A, Chiriboga A, Cortés J, Delbeek JC, Devantier L, Edgar GJ, Edwards AJ, Fenner D, Guzmán HM, Hoeksema BW, Hodgson G, Johan O, Licuanan WY, Livingstone SR, Lovell ER, Moore JA, Obura DO, Ochavillo D, Polidoro BA, Precht WF, Quibilan MC, Reboton C, Richards ZT, Rogers AD, Sanciangco J, Sheppard A, Sheppard C, Smith J, Stuart S, Turak E, Veron JE, Wallace C, Weil E

and Wood E (2008) One-third of reef-building corals face elevated extinction risk from climate change and local impacts. *Science* 321, 560.

Coles SL and Brown BE (2003) Coral bleaching – capacity for acclimatization and adaptation. *Advances in Marine Biology* 46, 183–223.

DeSalvo MK, Sunagawa S, Fisher PL, Voolstra CR, Iglesias-Prieto R and Medina M (2010) Coral host transcriptomic states are correlated with *Symbiodinium* genotypes. *Molecular Ecology* 19, 1174–1186.

Dimond JL, Holzman BJ and Bingham BL (2012) Thicker host tissues moderate light stress in a cnidarian endosymbiont. *Journal of Experimental Biology* 215, 2247–2254.

Dornelas M, Madin JS, Baird AH and Connolly SR (2017) Allometric growth in reef-building corals. *Proceedings of The Royal Society B, Biological Sciences* 284, 1851. https://doi.org/10.1098/rspb.2017.0053.

Dove SG, Lovell C, Fine M, Deckenback J, Hoegh-Guldberg O, Iglesias-Prieto R and Anthony KRN (2008) Host pigments: potential facilitators of photosynthesis in coral symbioses. *Plant, Cell & Environment* 31, 1523–1533.

Edgar RC (2004) MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* 5, 113.

Enríquez S, Méndez ER and Prieto RI (2005) Multiple scattering on coral skeletons enhances light absorption by symbiotic algae. *Limnology and Oceanography* 50, 1025–1032.

Fisher PL, Malme MK and Dove S (2012) The effect of temperature stress on coral–*Symbiodinium* associations containing distinct symbiont types. *Coral Reefs* 31, 473–485.

Franklin EC, Stat M, Pochon X, Putnam HM and Gates RD (2011) Geosymbio: a hybrid, cloud-based web application of global geospatial bioinformatics and ecoinformatics for *Symbiodinium*–host symbioses. *Molecular Ecology Resources* 12, 369–373.

Gajigan AP and Conaco C (2017) A microRNA regulates the response of corals to thermal stress. *Molecular Ecology* 26, 3472–3483.

Hoegh-Guldberg O, Poloczanska ES, Skirving W and Dove S (2017) Coral reef ecosystems under climate change and ocean acidification. *Frontiers in Marine Science* 4, 158.

Hoogenboom MO, Frank GE, Chase TJ, Jurriaans S, Álvarez-Noriega M, Peterson K, Critchell K, Berry KLE, Nicolet KJ, Ramsby B and Paley AS (2017) Environmental drivers of variation in bleaching severity of *Acropora* species during an extreme thermal anomaly. *Frontiers in Marine Science* 4, 376.

Hou J, Xu T, Su D, Wu Y, Cheng L, Wang J, Zhou Z and Wang Y (2018) RNA-Seq reveals extensive transcriptional response to heat stress in the stony coral *Galaxea fascicularis*. *Frontiers in Genetics* 9, 37.

Howells EJ, Berkelmans R, van Oppen MJH, Willis BL and Bay LK (2013) Historical thermal regimes define limits to coral acclimatization. *Ecology* 94, 1078–1088.

Hughes TP, Kerry JT, Álvarez-Noriega M, Álvarez-Romero JG, Anderson KD, Baird AH, Babcock RC, Beger M, Bellwood DR, Berkelmans R, Bridge TC, Butler IR, Byrne M, Cantin NE, Comeau S, Connolly SR, Cumming GC, Dalton SJ, Diaz-Pulido G, Eakin CM, Figueira WF, Gilmour JP, Harrison HB, Heron SF, Hoey AS, Hobbs JPA, Hoogenboom MO, Kennedy EV, Kuo CY, Lough JM, Lowe RJ, Liu G, McCulloch MT, Malcolm HA, McWilliam MJ, Pandolfi JM, Pears RJ, Pratchett MS, Schoepf S, Simpson T, Skirving WJ, Sommer B, Torda G, Wachenfeld DR, Willis BL and Wilson SK (2017) Global warming and recurrent mass bleaching of corals. *Nature* 543, 373.

Hume BC, D'Angelo C, Smith EG, Stevens JR, Burt J and Wiedenmann J (2015) *Symbiodinium thermophilum* sp. nov., a thermotolerant symbiotic alga prevalent in corals of the world's hottest sea, the Persian/Arabian Gulf. *Scientific Reports* 5, 8562.

Jokiel PL (2004) Temperature stress and coral bleaching. In Rosenberg E and Loya Y (eds), *Coral Health and Disease*. Berlin: Springer, pp. 401–425.

Keshavmurthy S, Meng PJ, Wang JK, Kuo CY and Yang SY (2014) Can resistant coral–*Symbiodinium* associations enable coral communities to survive climate change? A study of a site exposed to long-term hot water input. *PeerJ* 2, e327.

Knowlton N, Brainard RE, Fisher R, Moews M, Plaisance L and Caley MJ (2010) Coral reef biodiversity. In McIntyre AD (ed.), *Life in the World's Oceans*. Oxford: Wiley-Blackwell, pp. 65–78.

Krueger T, Hawkins TD, Becker S, Pontasch S, Dove S, Hoegh-Guldberg O, Leggat W, Fisher PL, and Davy SK (2015) Differential coral bleaching – contrasting the activity and response of enzymatic antioxidants in symbiotic

partners under thermal stress. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **190**, 15–25.

Kuguru B, Winters G, Beer S, Santos SR and Chadwick NE (2007) Adaptation strategies of the corallimorpharian *Rhodactis rhodostoma* to irradiance and temperature. *Marine Biology* **151**, 1287–1298.

LaJeunesse T (2002) Diversity and community structure of symbiotic dinoflagellates from Caribbean coral reefs. *Marine Biology* **141**, 387–400.

LaJeunesse TC and Trench RK (2000) Biogeography of two species of *Symbiodinium* (Freudenthal) inhabiting the intertidal sea anemone *Anthopleura elegantissima* (Brandt). *Biological Bulletin* **199**, 126–134.

LaJeunesse TC, Pettay DT, Sampayo EM, Phongsuwan N, Brown B, Obura DO, Hoegh-Guldberg O and Fitt WK (2010) Long-standing environmental conditions, geographic isolation and host–symbiont specificity influence the relative ecological dominance and genetic diversification of coral endosymbionts in the genus *Symbiodinium*. *Journal of Biogeography* **37**, 785–800.

LeGresley M and McDermott G (2010) Counting chamber methods for quantitative phytoplankton analysis – haemocytometer, Palmer-Maloney cell and Sedgewick-Rafter cell. In Karlson B, Cusack C and Bresnan E (eds), *Microscopic and Molecular Methods for Quantitative Phytoplankton Analysis*. Paris: UNESCO, pp. 25–30.

Lesser MP (1997) Oxidative stress causes coral bleaching during exposure to elevated temperatures. *Coral Reefs* **16**, 187–192.

Lesser MP (2006) Oxidative stress in marine environments: biochemistry and physiological ecology. *Annual Review of Physiology* **68**, 253–278.

Li S, Yu KF and Shi Q (2008) Experimental study of stony coral response to the high temperature in Luhuitou of Hainan Island. *Tropical Geography* **28**, 534–539.

Loya Y, Sakai K, Yamazato K, Nakano Y, Sambali H and van Woesik R (2001) Coral bleaching: the winners and the losers. *Ecology Letters* **28**, 534–539.

Marshall PA and Baird AH (2000) Bleaching of corals on the Great Barrier Reef: differential susceptibilities among taxa. *Coral Reefs* **19**, 155–163.

McPhaden MJ, Zebiak SE and Glantz MH (2006) ENSO as an integrating concept in Earth science. *Science* **314**, 1740.

Meyer E, Aglyamova GV and Matz MV (2011) Profiling gene expression responses of coral larvae (*Acropora millepora*) to elevated temperature and settlement inducers using a novel RNA-Seq procedure. *Molecular Ecology* **20**, 3599–3616.

Middlebrook R, Hoegh-Guldberg O and Leggat W (2008) The effect of thermal history on the susceptibility of reef-building corals to thermal stress. *Journal of Experimental Biology* **211**, 1050–1056.

Muscatine L and Porter JW (1977) Reef corals: mutualistic symbioses adapted to nutrient-poor environments. *BioScience* **27**, 454–460.

Oakley CA, Durand E, Wilkinson SP, Peng L, Weis VM, Grossman AR and Davy SK (2017) Thermal shock induces host proteostasis disruption and endoplasmic reticulum stress in the model symbiotic Cnidarian *Aiptasia*. *Journal of Proteome Research* **16**, 2121–2134.

Parkinson JE, Banaszak AT, Altman NS, LaJeunesse TC and Baums IB (2015) Intraspecific diversity among partners drives functional variation in coral symbioses. *Scientific Reports* **5**, 15667.

Peñaflor EL, Skirving WJ, Strong AE, Heron SF and David LT (2009) Sea-surface temperature and thermal stress in the Coral Triangle over the past two decades. *Coral Reefs* **28**, 841.

Putnam HM, Edmunds PJ and Fan T-Y (2010) Effect of a fluctuating thermal regime on adult and larval reef corals. *Invertebrate Biology* **129**, 199–209.

Ralph PJ, Hill R, Doblin MA and Davy SK (2015) Theory and application of pulse amplitude modulated chlorophyll fluorometry in coral health assessment. In Woodley CM, Downs CA, Bruckner AW, Porter JW and Galloway SB (eds) *Diseases of Coral*. Hoboken, NJ: John Wiley & Sons, pp. 506–523.

Richier S, Furla P, Plantivaux A, Merle PL and Allemand D (2005) Symbiosis-induced adaptation to oxidative stress. *Journal of Experimental Biology* **208**, 277–285.

Rodriguez-Lanetty M, Harii S and Hoegh-Guldberg O (2009) Early molecular responses of coral larvae to hyperthermal stress. *Molecular Ecology* **18**, 5101–5114.

Rohwer F, Seguritan V, Azam F and Knowlton N (2002) Diversity and distribution of coral-associated bacteria. *Marine Ecology Progress Series* **243**, 1–10.

Rowan R (2004) Coral bleaching: thermal adaptation in reef coral symbionts. *Nature* **430**, 742.

Salih A, Larkum A, Cox G, Kühl M and Hoegh-Guldberg O (2000) Fluorescent pigments in corals are photoprotective. *Nature* **408**, 850.

Sampayo EM, Dove S and Lajeunesse TC (2009) Cohesive molecular genetic data delineate species diversity in the dinoflagellate genus *Symbiodinium*. *Molecular Ecology* **18**, 500–519.

Silverstein RN, Cunning R and Baker AC (2015) Change in algal symbiont communities after bleaching, not prior heat exposure, increases heat tolerance of reef corals. *Global Change Biology* **21**, 236–249.

Stat M and Gates RD (2011) Clade D *Symbiodinium* in scleractinian corals: a "nugget" of hope, a selfish opportunist, an ominous sign, or all of the above? *Journal of Marine Biology* **730715**, 9.

Talavera G, Castresana J, Kjer K, Page R and Sullivan J (2007) Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic Biology* **56**, 564–577.

Tamura K, Stecher G, Peterson D, Filipski A and Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution* **30**, 2725–2729.

Traylor-Knowles N, Rose NH, Sheets EA and Palumbi SR (2017) Early transcriptional responses during heat stress in the coral *Acropora hyacinthus*. *Biological Bulletin* **232**, 91–100.

Truett GE, Heeger P, Mynatt RL, Truett AA, Walker JA and Warman ML (2000) Preparation of PCR-quality mouse genomic DNA with hot sodium hydroxide and tris (HotSHOT). *Biotechniques* **29**, 52, 54.

Veal CJ, Carmi M, Fine M and Hoegh-Guldberg O (2010) Increasing the accuracy of surface area estimation using single wax dipping of coral fragments. *Coral Reefs* **29**, 893–897.

Yakovleva I, Bhagooli R, Takemura A and Hidaka M (2004) Differential susceptibility to oxidative stress of two scleractinian corals: antioxidant functioning of mycosporine-glycine. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* **139**, 721–730.

Yamashita H, Suzuki G, Kai S, Hayashibara T and Koike K (2014) Establishment of coral-algal symbiosis requires attraction and selection. *PLoS ONE* **9**, e97003.

Yap HT, Espita DML, Montaño MNE, Benjamin C and Gomez ED (2014) Biochemical comparison of bleaching and non-bleaching *Montipora digitata* (Order Scleractinia) in the Philippines. *Philippine Science Letters* **7**, 293–299.

Yu K (2012) Coral reefs in the South China Sea: their response to and records on past environmental changes. *Science China Earth Sciences* **55**, 1217–1229.

Zar JH (1984) *Biostatistical Analysis*. London: Prentice Hall.