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Variation in bleaching sensitivity of two coral species across a latitudinal gradient on the Great Barrier Reef: the role of zooxanthellae

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ABSTRACT: The ability of corals to cope with environmental change, such as increased temperature, relies on the physiological mechanisms of acclimatisation and long-term genetic adaptation. We experimentally examined the bleaching sensitivity exhibited by 2 species of coral, *Pocillopora damicornis* and *Turbinaria reniformis*, at 3 locations across a latitudinal gradient of almost 6 degrees on the Great Barrier Reef (GBR). Target bleaching temperature was reached by using a ramping rate of 0.2°C/h. We found that the bleaching sensitivity and recovery of both species differed between corals with clade D symbionts and those with clade C. However, in *P. damicornis* bleaching susceptibility corresponded more strongly with latitude than with zooxanthella type and hence, temperature history, suggesting that local adaptation has occurred. The observed bleaching sensitivity was shown by a decrease in photochemical efficiency (F_v/F_m) in both species of coral. The rate of recovery in *T. reniformis* was highest in explants containing clade D symbionts. The occurrence of clade D in the northern section of the GBR may reflect a long-term response to high sea water temperatures, while the presence of clade D in low abundance in *T. reniformis* at Heralds Prong Reef and Percy Island may be a result of recent bleaching events.

KEY WORDS: Temperature tolerance · Corals · Bleaching · Zooxanthellae · *Symbiodinium* · F_v/F_m

INTRODUCTION

The symbiotic association between coral hosts and their algal endosymbionts, commonly known as zooxanthellae, is sensitive to increasing seawater temperatures combined with high irradiances (Muscatine 1990, Glynn 1996, Berkelmans & Oliver 1999, Hoegh-Guldberg 1999). The loss of zooxanthellae and/or photosynthetic pigments is known as coral bleaching and may cause coral death (Glynn & D'Croz 1990, Glynn et al. 2001) and reef degradation (Hoegh-Guldberg 1999). Incidences of mass coral bleaching have increased since the 1980s and was for the first time recorded on a world-wide scale in 1997-1998. The Great Barrier Reef (GBR) suffered most severely in the summer of 2002, when bleaching occurred on ~54% of its reefs (Berkelmans et al. 2004).

Bleaching susceptibilities are known to differ not only between coral taxa (Hoegh-Guldberg & Salvat 1995, Marshall & Baird 2000, Loya et al. 2001) but also between conspecific (Berkelmans & Oliver 1999, Berkelmans 2002) and congeneric (Coles et al. 1976) populations at geographically distinct locations. This suggests that thermal tolerance is governed by local environmental conditions to which corals have adapted (West & Salm 2003) or that have caused the expression of distinct phenotypes. The magnitude of differences in bleaching sensitivity of conspecific and geographically distinct coral populations has not previously been determined experimentally.

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It has been shown that the physiological responses of some corals are significantly affected by the type of zooxanthellae harboured (Rowan et al. 1997, Baker 2001, Glynn et al. 2001, Little et al. 2004, Rowan 2004). Furthermore, several studies have shown that some combinations of corals and their symbionts may be environmentally determined (Rowan et al. 1997, Toller et al. 2001, Ulstrup & van Oppen 2003, Baker et al. 2004, Fabricius et al. 2004). For example, the distribution of symbionts has shown clear patterns of photic zonation resulting in the partitioning of genetically distinct zooxanthellae between irradiance habitats in *Montastraea anularis* (Rowan et al. 1997) and *Acropora* spp. (van Oppen et al. 2001, Ulstrup & van Oppen 2003). Similar results have been found with regard to latitude (Loh et al. 2001, Rodriguez-Lanetty et al. 2001, Savage et al. 2002). It has also been shown that some symbiont genotypes are better suited to withstand elevated thermal exposure than others. For instance, Glynn et al. (2001) showed that *Pocillopora damicornis* colonies in the far-eastern Pacific harbouring clade D had suffered less from the 1998 mass coral bleaching event than those that associated with symbionts of a different genotype. Furthermore, clade D zooxanthellae inhabiting *Pocillopora* colonies showed higher photosystem activity than their clade C counterparts during elevated temperatures (Rowan 2004). These studies combined support the notion that clade D generally may be adapted to higher temperatures.

In this study, we examine the intra- and inter-colony distribution of zooxanthella types in 13 populations and the differences in temperature tolerances in 3 latitudinally distinct populations of 2 species of coral; 1 bleaching sensitive (*Pocillopora damicornis*) and 1 bleaching resistant (*Turbinaria reniformis*) species. We use variable chlorophyll *a* fluorescence (Jones et al. 1998, Ralph et al. 2001, Hill et al. 2004) as a measure of photoinactivation (decline in PSII activity), and zooxanthella density (Hoegh-Guldberg & Smith 1989, Berkelmans & Willis 1999, Glynn et al. 2001) to study heat stress responses in these corals. By comparing 2 coral species with contrasting bleaching sensitivity we hope to further our understanding of the processes and mechanisms involved in local adaptation to bleaching conditions.

MATERIALS AND METHODS

Examination of bleaching sensitivity was conducted on populations from the northern, central and southern section of the GBR (Fig. 1, Table 1). Only mid-shelf reefs were targeted to minimise any effects of cross-shelf position. However, additional genetic identification of zooxanthella communities was performed on a wider range of populations, including populations from inner- and mid-shelf locations (Fig. 1, Table 1).

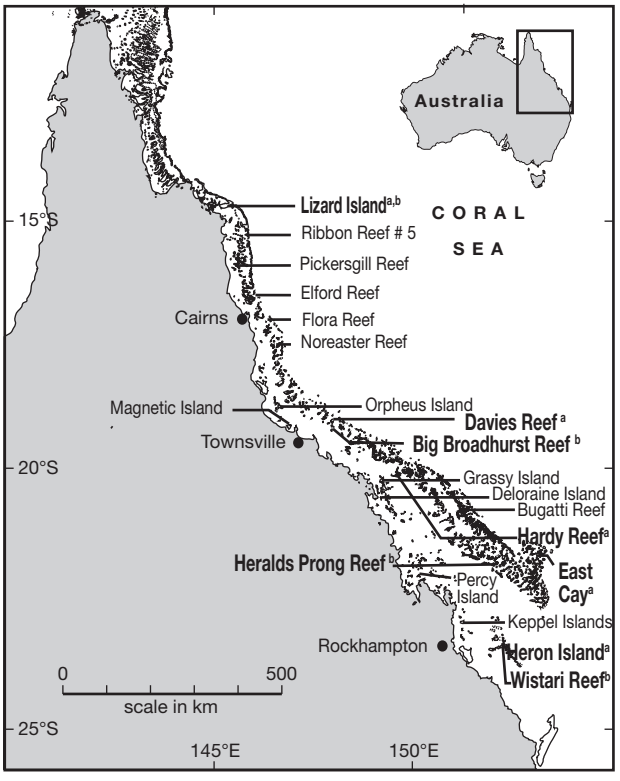

Fig. 1. Map of the Australian eastern sea border showing collection sites: [a]Locations of sea temperature measurements; [b]locations of experimental bleaching samples

Sampling. Nine coral colonies of each of *Pocillopora damicornis* and *Turbinaria reniformis* were sampled in August 2003 at Lizard Island (14.4° S, 144.3° E, northern GBR), Big Broadhurst Reef (18.3° S, 147.4° E, central GBR) and Wistari Reef (23.3° S, 151.5° E, southern GBR) (*P. damicornis* only). *P. damicornis* generally occurred and was sampled at depths between 1 and 10 m, whereas *T. reniformis* was sampled at depths between 3 and 15 m. *T. reniformis* could not be found at Wistari Reef and instead was sourced from Heralds Prong Reef (21.3° S, 151.2° E, southern GBR) (Fig. 1). Corals were maintained in flowing seawater (26°C) and transported to the Australian Institute of Marine Science (AIMS) in Townsville, where the bleaching sensitivity experiment was carried out.

Sea temperature measurements. To describe the relative climatology across a latitudinal gradient of the GBR, sea temperatures from 5 locations were obtained from the CRC (Cooperative Research Centre) Reef's long-term temperature monitoring program (www.reeffutures.org/topics/bleach/loggers.cfm). Daily averages were calculated from loggers (Dataflow Systems) deployed at 6 to 9 m depth over a 6 to 8 yr period be-

Table 1. *Pocillopora damicornis* and *Turbinaria reniformis*. Coral collection sites (see also Fig. 1), sample numbers (n), observed frequencies of zooxanthella type(s) present in both coral species (dominant clade in bold), and shelf position are given. Observed single-stranded conformation polymorphism genotype frequencies of clade A, C1, and D are f_A, f_{C1} and f_D, respectively

Sampling location		*P. damicornis*				*T. reniformis*			Shelf position
		n	f_A	f_{C1}	f_D	n	f_{C1}	f_D	
Northern GBR									
Lizard Island	Sun	10	**1**	10**(9)**	4	10	10	**10**	Mid
	Shade	10	**1**	10**(9)**	4	4	4	**4**	
Ribbon Reef # 5	Sun	10	–	**10**	2	2	2	**2**	Outer
	Shade	10	–	**10**	2	2	2	**2**	
Pickersgill Reef	Sun	10	–	**10**	–	7	7	**7**	Mid
	Shade	9	–	**9**	–	7	7	**7**	
Elford Reef	Sun	9	–	**9**	–	7	7	**7**	Mid
	Shade	10	–	**10**	–	10	10	**10**	
Flora Reef	Sun	8	–	**8**	–	8	8	**8**	Mid
	Shade	10	–	**10**	–	9	9	**9**	
Noreaster Reef	Sun	10	–	**10**	2	9	9	**9**	Mid
	Shade	10	–	**10**	2	10	10	**10**	
Central GBR									
Orpheus Island	Sun	10	–	**10**	–	–	–	**–**	Inner
	Shade	10	–	**10**	–	–	–	**–**	
Magnetic Island	Sun	10	–	**10**	–	10	**10**	**–**	Inner
	Shade	10	–	**10**	–	10	**10**	**–**	
Big Broadhurst Reef	Sun	8	–	**8**	3	9	**9**	**–**	Mid
	Shade	10	–	**10**	3	8	**8**	**–**	
Grassy Island	Sun	–	–	**–**	–	10	**10**	**–**	Inner
	Shade	–	–	**–**	–	10	**10**	**–**	
Deloraine Island	Sun	10	–	**10**	–	–	**–**	**–**	Inner
	Shade	9	–	**9**	–	–	**–**	**–**	
Bugatti Reef	Sun	7	–	**7**	–	10	**10**	**–**	Mid
	Shade	7	–	**7**	–	10	**10**	**–**	
Southern GBR									
Herald Prong Reef	Sun	10	–	**10**	–	8	**8**	6	Mid
	Shade	9	–	**9**	–	8	**8**	6	
Percy Island	Sun	10	–	**10**	–	10	10	**1**	Inner
	Shade	8	–	**8**	–	10	10	**1**	
Keppel Islands	Sun	10	–	**10**	–	10	**10**	**–**	Inner
	Shade	7	–	**7**	–	10	**10**	**–**	
Wistari Reef	Sun	9	–	**9**	–	–	**–**	**–**	Mid
	Shade	9	–	**9**	–	–	**–**	–	

tween 1995 and 2005 on the reef slope at Lizard Island (14° 41.3′ S, 145° 26.6′ E), Davies Reef (18° 48.4′ S, 147° 40.1′ E), Hardy Reef (19° 44.0′ S, 149° 10.0′ E) East Cay (21° 28.3′ S, 152° 34.0′ E) and Heron Island (23° 26.6′ S, 151° 54.7′ E). Davies Reef temperatures were recorded approximately 6 km from the collection site at Big Broadhurst Reef, while Hardy Reef is located approximately 300 km north and East Cay approximately 140 km east (i.e. same latitude), respectively, of our collection site at Heralds Prong Reef. Heron Island temperatures were recorded approximately 3 km from our collecting site at Wistari Reef. A 10 d smoothing function was applied to the data to highlight seasonal patterns. The temperature at collection sites corresponded to the averaged temperature observed in August as represented in Fig. 2.

Experimental procedure. The corals were placed in large indoor holding tanks with flow-through seawater

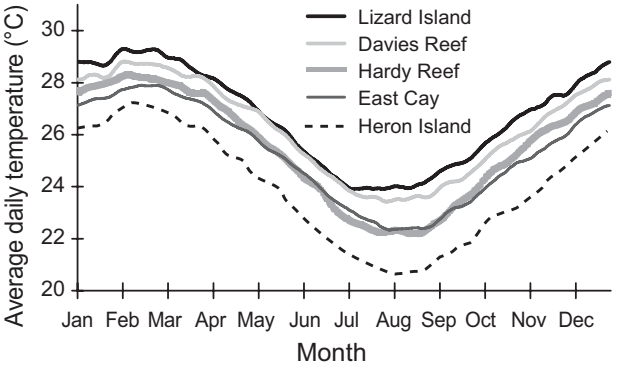

Fig. 2. Average daily temperatures for the reef slope (6 to 9 m depth) for each day (48 measurements per d) at Lizard Island, Davies Reef, Hardy Reef, East Cay and Heron Island. A 10 d smoothing function is applied to indicate the general trend in yearly temperatures

and acclimated to the control conditions for 2 wk prior to heating. Four experimental holding tanks, each containing ~1200 l of unfiltered seawater, were fitted with 2 heating elements (2.4 kw titanium, Thermal Electric Elements). Bleaching temperatures (29°C, 31°C, 33°C) were attained by ramping at 0.2°C/h. The 3 experimental bleaching temperatures and 1 control temperature (26°C) were maintained for 2 wk and subsequently experimental treatments were reduced to 26°C for recovery for an additional 2 wk. Temperature of the supply water was computer-controlled to target temperature ± 0.02 to 0.09°C SD (averaged and logged at 10 min intervals) (Turner et al. 2002). Temperature variation within the holding tanks was minimised by circulation of water (~2000 l h^{-1}) via submersible pumps (SICCE ULTRA 7000). Heated seawater was pumped from each holding tank into 3 replicate 35 l bins containing coral fragments. Metal halide lamps (400 watts:BLC, 10 000°K) provided a spectral output suitable for photosynthesis. Coral explants were exposed daily to approximately 300 µmol photons m^{-2} s^{-1} (LiCor) for 12 h followed by 12 h of darkness. One fragment from each of 9 *Pocillopora damicornis* and *Turbinaria reniformis* colonies from each of the 3 populations were represented in each bin.

Three additional samples of *Pocillopora damicornis* and *Turbinaria reniformis* from northern (Lizard Island), central (Big Broadhurst Reef) and southern (*P. damicornis*, Wistari Reef; *T. reniformis*, Heralds Prong Reef) GBR were placed in each of the 3 bins (n = 9) belonging to each of the 4 temperature treatments to verify the progression of bleaching by determining the density of zooxanthellae inside the coral tissue. One sample from each species in each of the bins was subsampled (n = 3) before heating (t = 0 d, n = 12), immediately after heating (t = 14 d, n = 3) and at the end of the recovery period (t = 28 d, n = 3). The approximate number of *Symbiodinium* cells in the samples was calculated from the average of 8 replicate haemocytometer counts and standardised to the liquid volume in which they were extracted and related to the coral surface area using the wax weight method described in Stimson (1997). The percentage change in zooxanthella density of explants exposed to 29 and 31°C in relation to those exposed to 26°C was calculated.

Single-point measurements of dark-adapted maximum quantum yield of PSII (F_v/F_m) (e.g. Schreiber 2004) were used to test the photosynthetic health (photochemical efficiency) of the zooxanthellae of all coral fragments (n = 27 per species per bin) throughout the pre-bleaching (data not shown), bleaching and recovery periods. Maximum quantum yield in corals (dark-adapted) was determined using a Diving-PAM fluorometer (Walz) after at least 8 h of darkness. Samples were illuminated with < 2 µmol photons m^{-2} s^{-1} during sampling. The percentage change in F_v/F_m of explants measured every 2 d was calculated in relation to pre-heating measurements. No samples were replaced in the statistical analysis. The assessment of death among explants was based on combined observations of fluorescence signals, lack of coral tissue movement and algal overgrowth.

Genetic identification of zooxanthellae in field colonies. Zooxanthellae harboured in sun- and shade-exposed tissues of 10 collected explants from each of the 3 experimental populations of both *Pocillopora damicornis* and *Turbinaria reniformis* were genetically identified. Only 9 of those were used in the bleaching sensitivity experiments. Hence, intra-colonial variability of zooxanthellae communities was assessed by sampling top and basal parts of colonies, which showed clear differences in colouration. Some colonies of *T. reniformis* did not show morphologies which had basal parts but were creeping partially up and under an overhang. In such cases, shaded samples were collected from these parts of the colonies.

To obtain a wider regional overview of symbiont types in these coral species, *P. damicornis* was collected from an additional 12 reefs and *T. reniformis* from an additional 10 reefs from north to south as follows: Ribbon Reef # 5 (15.2°S, 145.3°E), Elford Reef (16.6°S, 146.3°E), Flora Reef (17.1°S, 146.2°E), Noreaster Reef (17.5°S, 146.4°E), Orpheus Island (18°35′S, 146°20′E) (*P. damicornis*), Magnetic Island (19°15′S, 146°50′E), Grassy Island (20.1°S, 148.3°E), Deloraine Island (20.9°S, 149.4°E), Bugatti Reef (20.1°S, 150.2°E), Heralds Prong Reef (21.3°S, 151.2°E) (*P. damicornis*), Percy Island (21.4°S, 150.1°E), Keppel Islands (23.1°S, 150.6°E) (Fig. 1, Table 1). The sampling locations covered 1400 km in latitude as well as inner-, mid-, and outer shelf locations (Fig. 1, Table 1).

DNA was extracted from the corals using the DNeasy tissue extraction kit (Qiagen). The zooxanthella ribosomal DNA internal transcribed spacer 1 (ITS1) was PCR amplified as described in van Oppen et al. (2001), using a fluorescently labeled forward primer. Single-stranded conformation polymorphism (SSCP) (Sunnucks et al. 2000) analysis and DNA sequence analysis was used to determine the genetic identity of the zooxanthella samples. For SSCP analysis, amplicons were denatured at 98°C and snap-cooled on ice to allow the single-stranded DNA (ssDNA) to fold back onto itself. The products were run on a 4% non-denaturing polyacrylamide gel and visualized using the GelScan2000 system (Corbett Research) to obtain SSCP profiles. PCR products that resulted in different SSCP profiles were sequenced as described in van Oppen et al. (2001) and compared to existing sequences stored in Genbank (www.ncbi.nlm.nih.gov).

Statistical analyses. Comparisons of zooxanthella densities prior to, after heating and after recovery of latitudinally distinct populations of *Pocillopora damicornis* and *Turbinaria reniformis* were tested using 1-way ANOVA. Since all explants of both species died during the 2 weeks of exposure to 33°C, only samples which were exposed to 29°C and 31°C were statistically analysed. Assumptions of normality and of homogeneity of variances were tested using Smirnov-Komolgorov's and Levene's test, respectively. The non-parametric Kruskal–Wallis test was performed where normality and equal variance were not achieved.

Factorial univariate, repeated measured analysis of variance was performed on F_v/F_m measurements in order to determine the relationship of temperature treatments with latitudinally distinct populations of *Pocillopora damicornis* and *Turbinaria reniformis*. Day was analysed as within-subject factor as they were repeated measures on the same colony and Temp (temperature) and Location were analysed as between-subject factor (see Table 4 & 5). Bleaching and recovery periods were analysed separately. Where ANOVA determined a significant difference, Box's test of equality of covariance matrices was used to test the homogeneity of variances across groups. Also, the assumption of sphericity was tested. Where assumptions of the analyses were not met, the Greenhouse-Geisser adjustment was performed (Quinn & Keough 2002). Tukey's multiple comparisons were used to establish independency between treatments, populations and species. All analyses were performed using SPSS software v11.0.0.

RESULTS

Temperature range

Fig. 2 shows the daily sea water temperature averages for the reef slope (6 to 9 m depth) for each day of the year from Lizard Island, Davies Reef (our surrogate for Big Broadhurst Reef), Hardy Reef and East Cay (our surrogates for Heralds Prong Reef) and Heron Island (our surrogate for Wistari Reef). Although we were unable to obtain data in the near vicinity of Heralds Prong Reef it is a reasonable assumption that the temperature regime at this location would fall within the range of that observed at Heron Island, East Cay and Hardy Reef. The annual temperature variation corresponds with latitude. Heron Island shows the lowest winter and summer sea surface temperatures (20.7°C and 27.2°C, respectively), whereas Lizard Island shows the highest winter and summer daily average sea surface temperatures (23.8°C and 29.3°C, respectively). Although Davies Reef is located roughly equidistant from Lizard and Heron Island, its temperature range is close to that at Lizard Island (23.4 to 28.8°C), whereas Hardy Reef and East Cay exhibit average temperatures between those of Davies Reef and Heron Island (Fig. 2).

There was no significant variation between holding tanks containing 26°C seawater in the pre-heating and recovery phase ($p < 0.05$). Similarly, temperatures measured continuously in holding tanks during heating showed no overlap in calculated 95 percentiles (data not shown). Therefore, the variation within temperature treatments in the pre-heating, heating and recovery phase was within satisfactory precision.

Mortality

A comparison of accumulated mortality (%) between *Pocillopora damicornis* and *Turbinaria reniformis* confirmed that *P. damicornis* is the more bleaching sensitive species (Fig. 3). *P. damicornis* explants from Wistari Reef showed the earliest and severest mortality for all temperature treatments (Fig. 3A). At 29°C, initial mortality of Wistari Reef explants was encountered after 5 d (Fig. 3A), whereas initial mortality at 31 and 33°C occurred after 1 d (Fig. 3A). Complete mortality of experimental explants of *P. damicornis* was reached after 5 to 7 d at 33°C for corals from all locations (Fig. 3A). *P. damicornis* explants derived from Lizard Island kept at 31°C showed lowest total mortality and those at 33°C exhibited the most delayed onset of mortality (black bars, Fig. 3A). Mortality among *P. damicornis* explants continued to rise throughout the recovery period (Fig. 3A). The highest mortality during the recovery period occurred in Big Broadhurst Reef explants (22%) and Wistari Reef (29%) previously exposed to 31°C (Fig. 3A). Throughout the heating and recovery period, accumulated mortality among *P. damicornis* explants maintained at 26°C was 18 to 29% (Fig. 3A).

Mortality of *Turbinaria reniformis* was only observed among explants exposed to 33°C (Fig. 3B). Complete mortality (100%) was reached after 13 d of exposure to 33°C for all 3 locations (Fig. 3B). There was little difference in the relative increase in mortality between locations, although explants from Heralds Prong Reef suffered 22% more mortality than those from Lizard Island and Big Broadhurst Reef after 9 d of exposure to 33°C. No mortality occurred among *Turbinaria reniformis* explants during the recovery period following heating to 29 and 31°C (Fig. 3B), indicating that the heating period did not have a delayed effect.

Zooxanthella density

Pocillopora damicornis explants from Wistari Reef exhibited the highest ($p < 0.001$) zooxanthella density

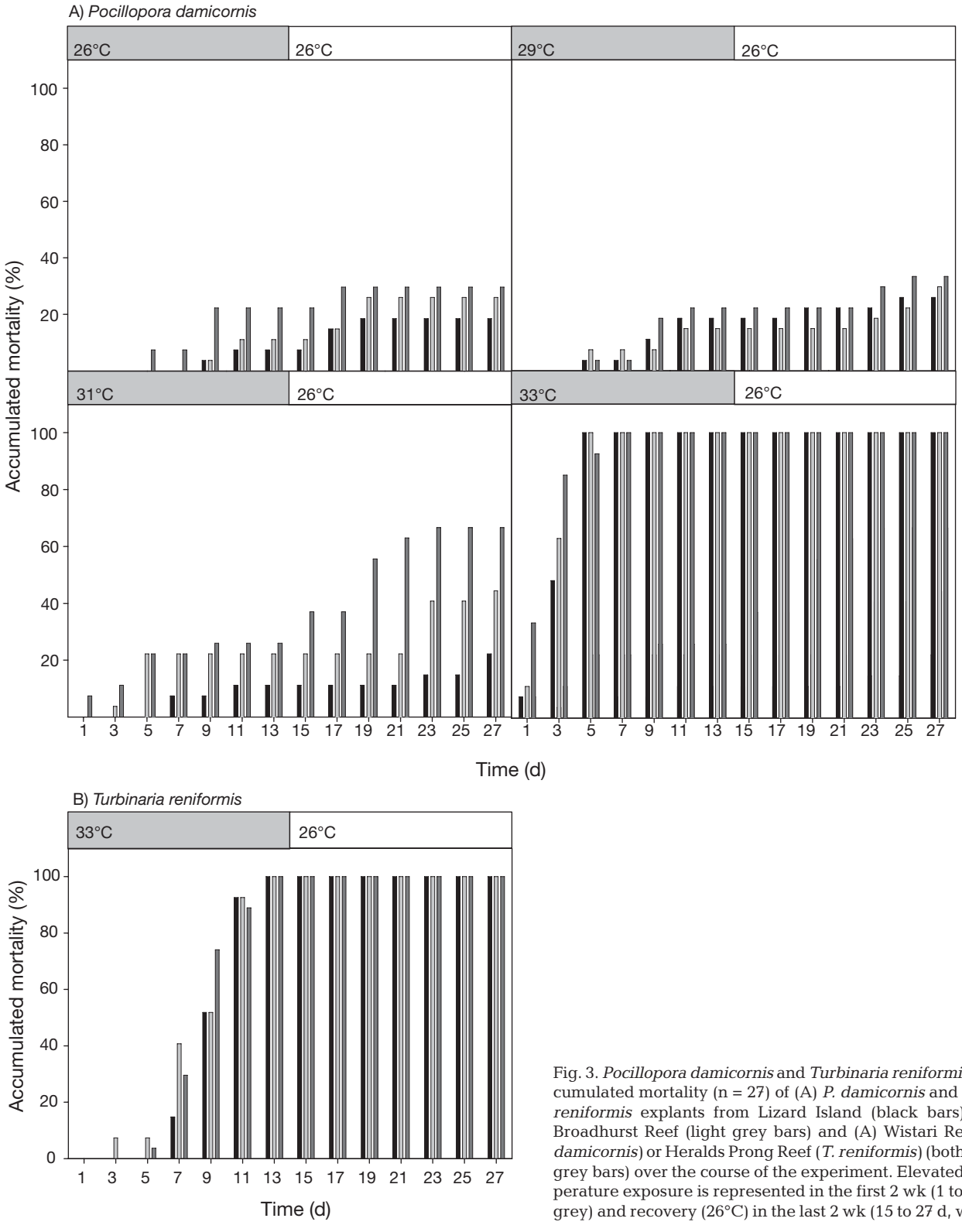

Fig. 3. *Pocillopora damicornis* and *Turbinaria reniformis*. Accumulated mortality (n = 27) of (A) *P. damicornis* and (B) *T. reniformis* explants from Lizard Island (black bars), Big Broadhurst Reef (light grey bars) and (A) Wistari Reef (*P. damicornis*) or Heralds Prong Reef (*T. reniformis*) (both dark grey bars) over the course of the experiment. Elevated temperature exposure is represented in the first 2 wk (1 to 13 d, grey) and recovery (26°C) in the last 2 wk (15 to 27 d, white)

followed by Lizard Island and Broadhurst Reef (Wistari Reef > Lizard Island > Big Broadhurst Reef; Table 2). Zooxanthella density of control explants of *P. damicornis* from Big Broadhurst Reef exposed to 26°C increased significantly ($p < 0.05$) throughout the experimental period (Table 2). No density counts are available for explants exposed to 33°C as they all died. *P. damicornis* explants from Wistari Reef showed a sig-

nificant decline in zooxanthella densities when exposed to 31°C, as well as after the following recovery period (Fig. 4A). No significant change in zooxanthella density was observed between the heating and recovery period for all geographically distinct populations exposed to both 29 and 31°C (Fig. 4A).

No decline in zooxanthella density in control explants of *Turbinaria reniformis* was observed during the experimental period (Table 2). The decline in zooxanthella density in *T. reniformis* explants from all populations was significant after exposure to 31°C (Fig. 4B). Zooxanthella density in Lizard Island explants following recovery from 29°C was significantly greater ($p < 0.05$) than before experimental elevation of the temperature (Fig 4B). Of all explants exposed to 31°C only those from Lizard Island showed zooxanthella densities similar to pre-heating levels (Fig. 4B). Heralds Prong Reef explants exposed to 31°C showed the greatest decline in zooxanthella density including those measured after the recovery period.

Table 2. Zooxanthella density (numbers of cells × 10^6 cm^{-2} surface area, ± SE) of controls (26°C) prior to experimental heating (*t* (d) = 0: 1 replicate in each of 3 bins of 4 experimental temperature tanks, n = 12), after heating (*t* = 14: 1 replicate in each of 3 bins, n = 3) and after recovery (*t* = 28: 1 replicate in each of 3 bins, n = 3) in northern (Lizard Island), central (Big Broadhurst Reef) and southern (*Pocillopora damicornis*, Wistari Reef; *Turbinaria reniformis*, Heralds Prong Reef) GBR. Asterisk indicates statistical significance. ns: not significant; –: not analysed

	t (d)	Northern GBR	Central GBR	Southern GBR	p-value
P. damicornis	0	0.54 ± 0.06	0.32 ± 0.05	1.01* ± 0.01	<0.001
	14	0.52 ± 0.01	0.36 ± 0.03	0.53 ± 0.02	–
	28	0.43 ± 0.09	0.91* ± 0.08	0.53 ± 0.03	–
p-value		ns	<0.05	ns	
T. reniformis	0	0.59 ± 0.08	0.45 ± 0.03	0.43 ± 0.05	ns
	14	0.54 ± 0.03	0.42 ± 0.01	0.31 ± 0.08	–
	28	0.37 ± 0.04	0.31 ± 0.03	0.35 ± 0.03	–
p-value		ns	ns	ns	

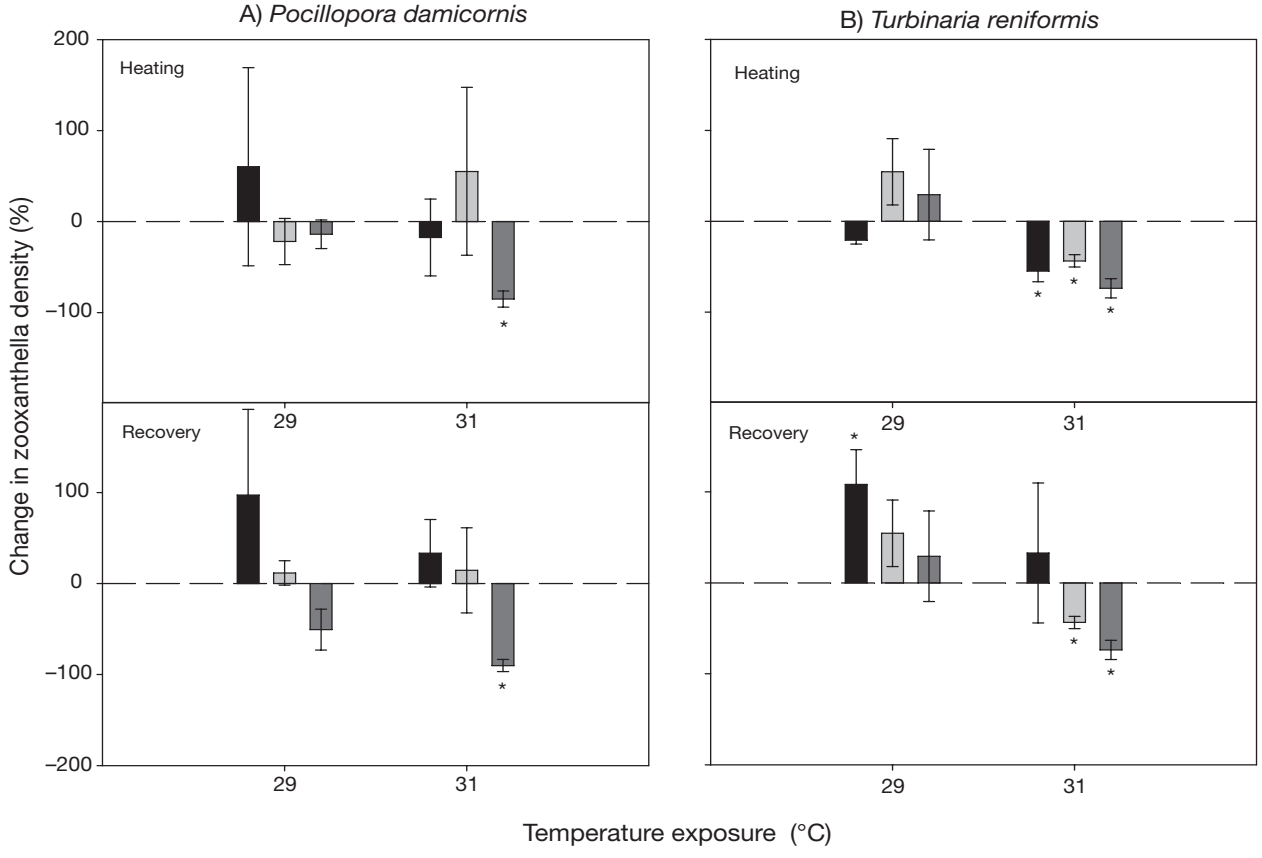

Fig. 4. *Pocillopora damicornis* and *Turbinaria reniformis*. Relative change (%) of zooxanthella density (10^6 cm^{-2}) in relation to controls of (A) *P. damicornis* and (B) *T. reniformis* explants from Lizard Island (black bars), Big Broadhurst Reef (light grey bars) and Wistari Reef (*P. damicornis*) or Heralds Prong Reef (*T. reniformis*) (both dark grey bars) after 2 wk of exposure to elevated temperatures (29 and 31°C) and 2 wk of recovery. Significant differences were calculated by testing between sites at specific temperatures. Averages are shown (n = 3) including ±SE bars. Significant differences ($p < 0.05$) are marked by an asterisk

Photochemical efficiency (F_v/F_m)

Pocillopora damicornis

No significant differences were observed in initial F_v/F_m measurements between populations (Table 3). Control explants (26°C) showed variable change in F_v/F_m over time ranging between 2 and 29% during the 4 experimental wk (Fig. 5A[a]). No significant interactions between day and location were observed in the change of F_v/F_m measurements over time ($p = 0.087$, Table 4a) suggesting that the origin of explants did not influence the rate of bleaching in *Pocillopora damicornis*. However, the 3-way interaction between day, temperature and location ($p < 0.001$, Table 4a) and between subject analysis of temperature ($p < 0.001$, Table 4a) suggest that the experimental temperature exposure does influence decline in F_v/F_m measurements. Post hoc analysis identified differences in F_v/F_m measurements when explants were exposed to 31 and 33°C. Between subject analysis of location showed significant differences at these temperatures. Thus, post hoc analysis identified explants from Wistari Reef as being most sensitive to high temperature exposure (5A[c,d]). Explants from Lizard Island and Big Broadhurst Reef showed slower initial declines in F_v/F_m than explants from Wistari Reef. However, explants from Lizard Island were the first to bleach completely after 5 d, whereas explants from Big Broadhurst and Wistari Reefs resisted complete bleaching for 7 d (Fig. 5A[d]).

Between subject analysis showed that the decline in F_v/F_m observed during the recovery period correlated with previous temperature exposure ($p < 0.001$, Table 4b). The continual decline during the recovery period of explants exposed to 31°C (Fig. 5A[c]) was greatest in Wistari Reef explants ($p < 0.001$, Table 4a). Thus, at 31°C the sequence of bleaching susceptibility between *Pocillopora damicornis* populations was Wistari Reef > Big Broadhurst Reef > Lizard Island as indicated by the level of F_v/F_m at the end of the recovery period (Fig. 5A[c]).

Table 3. Absolute photochemical efficiency (F_v/F_m ± 1 SE; n = 27) prior to experimental heating of explants in northern (Lizard Island), central (Big Broadhurst Reef) and southern (*Pocillopora damicornis*, Wistari Reef; *Turbinaria reniformis*, Heralds Prong Reef) GBR. Values with different superscript letters were significantly different; ns: not significant

	Northern GBR	Central GBR	Southern GBR	p-value
P. damicornis	0.684 ± 0.008	0.667 ± 0.008	0.663 ± 0.006	ns
T. reniformis	0.606^a ± 0.004	0.574^b ± 0.009	0.635^c ± 0.007	<0.01

Turbinaria reniformis

All 3 populations showed initial significant differences (< 0.01) between populations in F_v/F_m (Table 3). Control explants (26°C) showed minimal change (both increases and decreases) in F_v/F_m over time ranging between 4 and 8% during the 4 experimental

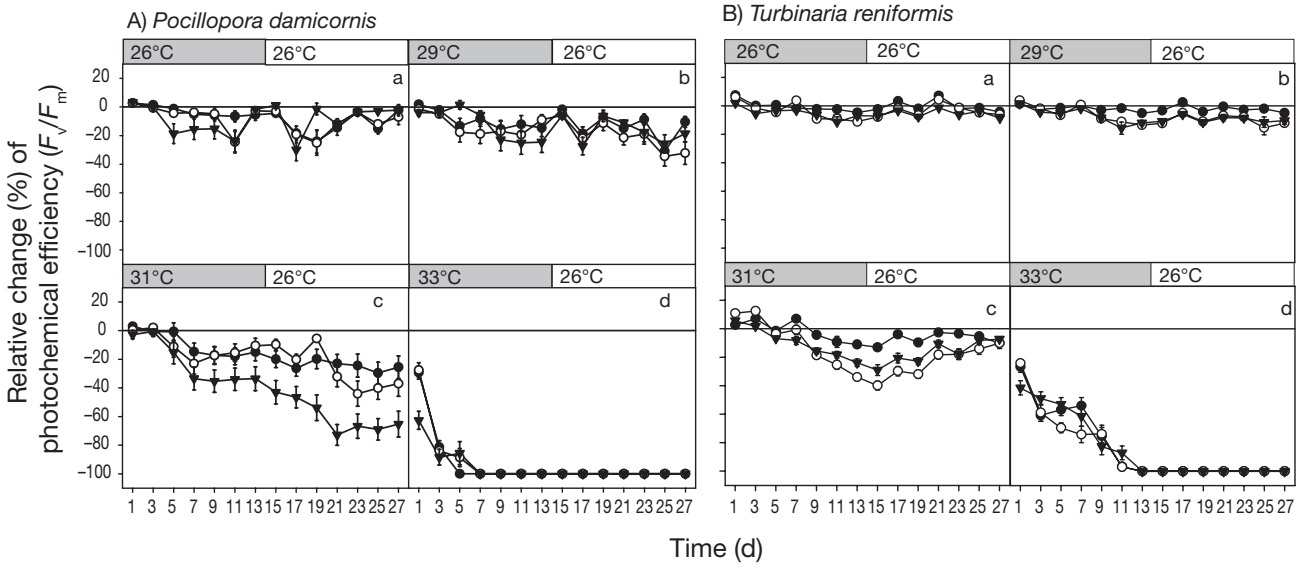

Fig. 5. *Pocillopora damicornis* and *Turbinaria reniformis*. Average relative change (%) of photochemical efficiency (F_v/F_m) in relation to pre-bleaching responses (n = 27 ± 1 SE) of (A) *P. damicornis* and (B) *T. reniformis* explants from Lizard Island (solid circles), Big Broadhurst Reef (white circles) and Wistari Reef (*P. damicornis*) or Heralds Prong Reef (*T. reniformis*) (both triangles). Two wk of (a) control and (b–d) bleaching treatments are shown (1 to 13 d, grey) followed by 2 wk of recovery (15 to 27 d, white)

Table 4. Univariate analyses of (a) heating and (b) recovery of *Pocillopora damicornis* testing the hypothesis that changes in F_v/F_m following a change in temperature are independent of sampling origin. Day (d, within-subject factor): 0, 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27 ; temperature (between-subject factor): 26, 29, 31, 33°C; location (between-subject factor): LI = Lizard Island, BBR = Big Broadhurst Reef, WR = Wistari Reef

Source of variation	SS	df	df^{adj}	MS	*F*	p[a]	Tukey's
a) ANOVA for repeated measures							
Within subjects							
Day	23.494	7	5.402	4.349	171.043	<0.001	d1>d3>d9
Day × Location	0.446	14	10.803	4.132×10^{-2}	1.625	0.087	
Day × Temp	22.733	21	16.205	1.403	55.169	<0.001	
Day × Temp × Location	2.176	42	32.409	6.713×10^{-2}	2.640	<0.001	
Between subjects							
Temp	97.519	3		32.506	727.665	<0.001	26°C, 29°C>31°C >33°C
Location	1.270	2		0.635	14.210	<0.001	LI, BBR>WR
Temp × Location	345	6		5.753×10^{-2}	1.288	0.262	
b) ANOVA for repeated measures							
Within subjects							
Day	2.268	7	5.739	0.395	13.291	<0.001	d15>d17>d23>d25>d27
Day × Location	0.524	14	11.478	4.567×10^{-2}	1.535	0.109	
Day × Temp	3.520	21	17.217	0.204	6.870	<0.001	
Day × Temp × Location	1.616	42	34.434	4.694×10^{-2}	1.577	0.018	
Between subjects							
Temp	149.893	3		49.964	924.336	<0.001	26°C>29°C>31°C
Location	1.657	2		0.828	15.323	<0.001	LI, BBR>WR
Temp × Location	16.865	6		0.894	16.531	<0.001	

[a]Lower bound p-values are calculated by adjusting the degrees of freedom for the test by multiplying by the smallest possible Greenhouse-Geisser adjustment (Quinn & Keough 2002)

weeks (Fig. 5B[a]). Significant 2- and 3-way interactions were observed between day, location and temperature during bleaching and recovery ($p < 0.001$, Table 5a,b). Between subject analysis and Tukey's post hoc test showed that changes in F_v/F_m over time of 26 and 29°C was similar, whereas 31 and 33°C showed progressively greater declines of F_v/F_m ($p < 0.001$, Table 5a). After 9 d, Big Broadhurst and Heralds Prong Reef explants showed a greater decline than Lizard Island explants (Fig. 5B[c]). By the end of the 31°C exposure period, Big Broadhurst Reef explants showed the greatest decline followed by Heralds Prong Reef explants (Fig. 5B[c], Table 5a). All *Turbinaria reniformis* explants survived heating to 33°C for 11 d (Fig. 5B[d]) and thus showed a less rapid decline in F_v/F_m compared to *Pocillopora damicornis* (Fig. 5A[d]).

No further decline in F_v/F_m of *Turbinaria reniformis* explants exposed to 26, 29 and 31°C was observed during recovery (Fig. 5B[a–c]). Between subject analysis of temperature showed a significant difference between recovery at different temperatures. Tukey's post hoc test identified the recovery as being correlated to previous temperature exposure ($p < 0.001$, Table 5b). Explants from Lizard Island showed the most rapid recovery followed by explants from Heralds Prong Reef ($p < 0.001$, Table 5b, Fig. 5B[c]).

Genotyping of zooxanthellae

The results show that colonies used to examine bleaching sensitivity harboured predominantly *Symbiodinium* strain C1, except for *Turbinaria reniformis* explants from Lizard Island which were dominated by *Symbiodinium* clade D. *Pocillopora damicornis* colonies collected from all sites along the latitudinal gradient were dominated by *Symbiodinium* clade C, specifically, the C1 strain as described in van Oppen et al. (2001), GenBank Accession # AY457958 (Table 1). The faint gel bands observed in this study using SSCP analysis represented a minimum of 5 to 10% relative abundance of zooxanthella strains present in the sample (Fabricius et al. 2004). Thus, *Symbiodinium* clade D (Genbank Accession # AY327073) co-occurred with clade C in upper and lower surfaces of 2 colonies from Noreaster and Ribbon Reef # 5, 3 colonies from Big Broadhurst Reef and 4 colonies from Lizard Island. One *P. damicornis* colony at Lizard Island was found to harbour clade A zooxanthellae in both upper and lower surfaces in addition to clade C. In this colony, clade A (GenBank Accession # AF380513) was the dominant type. None of the sampled colonies exhibited any intra-colony differences in zooxanthella types between upper and lower surfaces.

Table 5. Univariate analyses of (a) heating and (b) recovery of *Turbinaria reniformis* testing the hypothesis that changes in F_v/F_m following a change in temperature are independent of sampling origin. Day (d, within-subject factor): 0, 1, 3, 5, 7, 9, 11 13, 15, 17, 19, 21, 23, 25, 27; temperature (between-subject factor): 26, 29, 31, 33°C; location (between-subject factor): LI = Lizard Island, BR = Big Broadhurst Reef, HPR = Heralds Prong Reef

Source of variation	SS	df	df^{adj}	MS	*F*	p[a]	Tukey's
a) ANOVA for repeated measures							
Within subjects							
Day	14.720	7	4.661	3.158	302.277	<0.001	d1>d5>d7>d9>d11>d13
Day × Location	0.376	14	9.322	4.037×10^{-2}	3.864	<0.001	
Day × Temp	16.078	21	13.984	1.150	110.054	<0.001	
Day × Temp × Location	1.011	42	27.967	3.614×10^{-2}	3.459	<0.001	
Between subjects							
Temp	46.624	3		15.541	1106.12	<0.001	26°C, 29°C>31°C >33°C
Location	1.114	2		0.557	39.654	<0.001	LI, HPR>BBR
Temp × Location	0.257	6		4.283×10^{-2}	3.048	<0.001	
b) ANOVA for repeated measures							
Within subjects							
Day	0.538	7	5.312	0.101	33.596	<0.001	d15<d17<d19<d25<d27
Day × Location	0.115	14	10.625	1.084×10^{-2}	3.596	<0.001	
Day × Temp	0.779	21	15.937	4.889×10^{-2}	16.225	<0.001	
Day × Temp × Location	0.245	42	31.874	7.682×10^{-3}	2.549	<0.001	
Between subjects							
Temp	148.110	3		49.370	5009.194	<0.001	26°C>29°C>31°C
Location	1.850	2		0.925	93.865	<0.001	LI >HPR>BBR
Temp × Location	1.085	6		0.181	18.353	<0.001	

[a]Lower bound p-values are calculated by adjusting the degrees of freedom for the test by multiplying by the smallest possible Greenhouse-Geisser adjustment (Quinn & Keough 2002)

The 6 northernmost populations of *Turbinaria reniformis* (Lizard Island, Ribbon Reef # 5, Pickersgill Reef, Elford Reef, Flora Reef, Noreaster Reef) were dominated by clade D (GenBank Accession # AY327073) zooxanthellae, with *Symbiodinium* C1 being present at low abundances in both upper and lower parts of all colonies. At Percy Island, all *T. reniformis* colonies harboured predominantly strain C1 except for one which harboured mainly clade D. At Heralds Prong Reef all colonies harboured mainly strain C1 but in 6 of the colonies low abundances of clade D were also detected. *Symbiodinium* C1 was the only detectable zooxanthella type in colonies from all other sites. The distribution of zooxanthellae did not correspond with shelf position (Table 1).

DISCUSSION

In this study, we have documented intra-specific differences in bleaching sensitivity of 2 coral species (*Pocillopora damicornis* and *Turbinaria reniformis*) from 3 geographically distinct locations along a latitudinal gradient of the GBR. The 2 coral taxa show distinct depth distributions (K. E. Ulstrup, R. Berkelmans, M. J. H. van Oppen pers. obs.) and are also likely to possess distinct host attributes not assessed in this study, which may influence their thermal tolerance. The results show that the temperature history (Fig. 2) and geographic distribution of zooxanthellae is of great importance to the bleaching sensitivity of corals and that even very bleaching resistant corals such as *T. reniformis* are photochemically sensitive to heat stress (Fig. 5B). Despite the fact that the coral genus *Turbinaria* has been shown to be one of the least susceptible taxa during natural bleaching events (Marshall & Baird 2000), our experimental results show that the difference in susceptibility between *T. reniformis* and a sensitive species like *P. damicornis* is less than 2°C and may even be less than 1°C for a 2 wk exposure. However, it should be noted that the rate of thermal ramping of 0.2°C/h is faster than what corals are likely to experience in a natural setting, which may have influenced the measured difference in bleaching susceptibility between these 2 species.

We have shown genetic differences in the geographic distribution of the zooxanthella communities in *Pocillopora damicornis* and *Turbinaria reniformis*. The bleaching sensitivity of both *P. damicornis* and *T. reniformis* corresponded with the relative presence and dominance of clade D zooxanthellae. Although bleaching sensitivity in *P. damicornis* also corresponds to latitude, this does not appear to be the case for *T. reniformis*. This result reinforces the notion that

temperature tolerance in some coral species may be driven by symbiont type and perhaps to a lesser extent by host biochemistry. Thus, this study provides support to functional differences existing among zooxanthella types whose traits are conferred upon the physiology of the coral host.

Local adaptation and phenotypic plasticity

Pocillopora damicornis has been affected by bleaching worldwide and has often served as an experimental species for studies of coral physiology in relation to bleaching (Glynn & D'Croz 1990, D'Croz & Maté 2004, Hill et al. 2004, Ralph et al. 2005). Glynn & D'Croz (1990) found colonies of *P. damicornis* from an upwelling area in the Gulf of Panama to undergo greater bleaching at 30°C in controlled experiments than the same species from the non-upwelling Gulf of Chiriqui, where ambient temperatures were higher and more stable. Our data confirm that *P. damicornis* colonies are highly sensitive to elevated temperatures and that adaptation to local temperatures (Fig. 2) influences their bleaching resistance, as latitudinal differences in bleaching sensitivity were maintained after laboratory acclimation. Thus, corals pre-exposed to high temperature (i.e. low latitude: Lizard Island) were less sensitive to the experimental temperature treatments and corals accustomed to relatively lower temperature (i.e. high latitude: Wistari Reef) bleached earlier and more severely (Fig. 5A). Lizard Island and Ribbon # 5 in the northern section of the GBR and Big Broadhurst Reef in the central section of the GBR showed low abundance of clade D in some explants (Table 1) suggesting potential capacity for symbiont shuffling (i.e. changes in the relative abundance of different zooxanthella types present inside the host tissues (Baker 2003)) in response to elevated temperatures. The pattern however is not consistent throughout the northern section of the GBR suggesting that factors other than temperature are driving symbiont selection.

The populations of *Turbinaria reniformis* showed less sensitivity to elevated temperature than *Pocillopora damicornis* (Fig. 5). Temperature resistance in *T. reniformis* corresponded with the presence of clade D symbionts (Table 1). The most bleaching resistant population (Lizard Island) showed dominance of clade D symbionts, followed by explants from Heralds Prong Reef which harboured clade D symbionts at a lower abundance than strain C1 and explants from Big Broadhurst Reef which did not show any presence of clade D symbionts (Table 1). This suggests that clade D symbionts are selected for in populations exposed to high temperatures, such as found in the northern GBR. Baker et al. (2004) found that the abundance of clade D symbionts was high at locations where recent bleaching had occurred. Also, van Oppen et al. (2005) found that several *Acropora millepora* populations in the southern section of the GBR, which had bleached in 2002, showed an increased abundance of clade D since this bleaching event. The co-occurrence of genetically distinct symbionts in *T. reniformis* among reefs on the southern GBR (Heralds Prong Reef and Percy Island, Table 1) might thus be due to past bleaching events on these reefs.

Mortality

Both species showed 100% mortality when exposed to 33°C (*Pocillopora damicornis* after 5 to 7 d depending on location and *Turbinaria reniformis* after 13 d) (Fig. 3). Persistent exposure to 33°C was beyond the bleaching resistance capacity of both corals in our experiment. However, corals may show greater resistance during natural thermal events, during which thermal maxima are achieved more slowly, providing greater time for acclimatization of the holobiont. The corals were collected in the austral winter. It is possible that corals show greater resistance to heat stress if they are experimentally tested in summer when acclimated to higher temperature (Brown et al. 2002). Indeed, Berkelmans & Willis (1999) found that the winter bleaching threshold of *P. damicornis* on the GBR was 1°C lower than the summer threshold for this species and proposed that the winter temperature bleaching threshold of 31 to 32°C was a reliable predictor of subsequent mortality.

Zooxanthella density

Significant declines in zooxanthella densities were not observed after exposure to 29°C in either species from any of the populations examined (Fig. 4). However, the variation in zooxanthella density was generally greater in *Pocillopora damicornis* explants (Fig. 4A) than in *Turbinaria reniformis* explants (Fig. 4B). This suggests that the number of replicates examined was insufficient to detect differences which might have been present between control and experimental explants.

Corals have been found to exhibit increased host respiration in relation to photosynthesis at lower sea temperatures (Coles & Jokiel 1977). In order to compensate for loss of photosynthates, corals may increase the number of zooxanthellae inside their tissues. Prior to experimental treatment the highest zooxanthella densities in *Pocillopora damicornis* were found at Wistari

Reef (Table 2). Wistari Reef explants also showed the largest relative decrease in zooxanthella density as observed after exposure to 31°C (Fig. 4A), possibly due to their thermal history of lower temperatures (Fig. 2) than explants from Big Broadhurst Reef and Lizard Island.

All populations of *Turbinaria reniformis* were sensitive to exposure to 31°C but only explants from Lizard Island returned to pre-bleaching levels of zooxanthella density during recovery (Fig. 4B). This possibly reflects the degree of damage incurred during the period of exposure to elevated temperatures, which was not as severe in explants from Lizard Island as in explants from Big Broadhurst Reef and Heralds Prong Reef as indicated by measurements of photochemical efficiency (F_v/F_m; Fig. 5B).

Photochemical efficiency

Decreased photochemical efficiency (F_v/F_m) is an indication of PSII inactivation and has typically been found to follow the onset of bleaching (Hoegh-Guldberg & Smith 1989). Both species of coral showed a decline in F_v/F_m over 14 d of elevated temperature at 31 and 33°C relative to that observed at the control temperature (Fig. 5). However, the variance in photochemical decline was greater for *Pocillopora damicornis* (Fig. 5A) than for *Turbinaria reniformis* (Fig. 5B) indicating less intra-specific stability of the holobiont. During the recovery period after exposure to 31°C, the F_v/F_m response of *P. damicornis* colonies from Big Broadhurst Reef and Wistari Reef continued to decline (Fig. 5A[c]), whereas *T. reniformis* colonies showed signs of recovery after 3 d and were able to revert the photoinactivation observed during the period of elevated temperature exposure (Fig. 5B[c]). The duration of bleaching may cause a bleaching sensitive species such as *P. damicornis* to lose its ability to recover due to irreparable damage to the PSII (Hill et al. 2004). However, a prolonged recovery period could result in an increase in the F_v/F_m signal as the photosynthetic apparatus is repaired (Warner et al. 1999).

Symbiont selection

Pocillopora damicornis explants were all dominated by *Symbiodinium* type C1. The presence of similar *Symbiodinium* communities in geographically distinct populations of *P. damicornis* suggests that this coral-algal association is physiologically compatible with near average summer temperature conditions. This specificity may be advantageous during normal conditions but may also be sub-optimal during extreme temperatures caused by climate change. Limited gene flow from *P. damicornis* populations adapted to different temperature regimes (Ayre & Hughes 2000) may drive populations to relatively higher temperature tolerance through local adaptation. None of the *P. damicornis* populations exhibited dominance of the more heat tolerant *Symbiodinium* clade D. However, a small number of *P. damicornis* colonies at 3 out of 6 northern sites and Big Broadhurst Reef in the central section of the GBR exhibited low abundances of clade D in relation to strain C1 (Table 1). Thus, the presence of clade D does not correspond well with latitude, which suggests that *P. damicornis* on the GBR exhibits limited plasticity in its selection for symbionts.

The distribution of genetically distinct symbionts in experimental populations of *Turbinaria reniformis* corresponds with F_v/F_m measurements, suggesting that clade D symbionts are physiologically more robust than C1 symbionts during exposure to elevated temperatures (Table 1, Fig. 5). Given that clade D symbionts have been found to be thermo-tolerant (Glynn et al. 2001, Baker et al. 2004, Fabricius et al. 2004, Rowan 2004), reef regions containing this group of symbionts may form source populations for the colonisation of disturbed reefs. Only very low levels of gene flow have been found to be necessary to permit the spread of advantageous genotypes (Morjan & Rieseberg 2004). The spread of clade D symbionts could thus provide a means to withstand severe future reef degradation due to bleaching in corals which exhibit flexibility in their symbiont preference, although an ecological cost may be associated with shuffling to a different type of zooxanthella (Little et al. 2004).

CONCLUSIONS

We found that the bleaching sensitivity and recovery of *Pocillopora damicornis* corresponded with latitude i.e. high-latitude corals bleached prior to and at lower temperatures than low-latitude corals. The 2 coral taxa showed different affinities for distinct zooxanthella genotypes. Although the northern and central experimental populations of *P. damicornis* harboured low abundances of clade D in some explants, we believe that the observed latitudinal difference in bleaching sensitivity suggests that the role of symbiont shuffling is inferior to that of local adaptation in this species. In contrast, the bleaching response of *Turbinaria reniformis* did not correspond with latitude but with the presence of thermo-tolerant clade D symbionts. Thus, corals which did not harbour detectable levels of *Symbiodinium* clade D were most sensitive to temperature elevation, irrespective of local temperature regimes.

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