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The effect of thermal history on the susceptibility of reef-building corals to thermal stress

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SUMMARY

The mutualistic relationship between corals and their unicellular dinoflagellate symbionts (*Symbiodinium* sp.) is a fundamental component within the ecology of coral reefs. Thermal stress causes the breakdown of the relationship between corals and their symbionts (bleaching). As with other organisms, this symbiosis may acclimate to changes in the environment, thereby potentially modifying the environmental threshold at which they bleach. While a few studies have examined the acclimation capacity of reef-building corals, our understanding of the underlying mechanism is still in its infancy. The present study focused on the role of recent thermal history in influencing the response of both corals and symbionts to thermal stress, using the reef-building coral *Acropora aspera*. The symbionts of corals that were exposed to 31°C for 48 h (pre-stress treatment) 1 or 2 weeks prior to a 6-day simulated bleaching event (when corals were exposed to 34°C) were found to have more effective photoprotective mechanisms. These mechanisms included changes in non-photochemical quenching and xanthophyll cycling. These differences in photoprotection were correlated with decreased loss of symbionts, with those corals that were not prestressed performing significantly worse, losing over 40% of their symbionts and having a greater reduction in photosynthetic efficiency. These results are important in that they show that thermal history, in addition to light history, can influence the response of reef-building corals to thermal stress and therefore have implications for the modeling of bleaching events. However, whether acclimation is capable of modifying the thermal threshold of corals sufficiently to cope as sea temperatures increase in response to global warming has not been fully explored. Clearly increases in sea temperatures that extend beyond 1–2°C will exhaust the extent to which acclimation can modify the thermal threshold of corals.

Supplementary material available online at http://jeb.biologists.org/cgi/content/full/211/7/1050/DC1

Key words: acclimation, thermal stress, *Symbiodinium sp.*, photoprotective mechanisms, coral bleaching.

INTRODUCTION

Average global sea temperatures have increased approximately 0.6°C since the beginning of the last century and are projected to increase by a further 1.1–6.4°C within the next 100 years (IPCC, 2007). This increase is expected to have substantial impacts on coral reefs and the tens of thousands of organisms that inhabit them (Hoegh-Guldberg, 1999; Hughes et al., 2003). Comparisons of known thermal tolerance levels for corals with projections of sea temperature changes strongly suggest that the frequency and intensity of coral bleaching and mortality is set to increase sharply, leading to declines in coral reefs worldwide (Hoegh-Guldberg, 1999; Hoegh-Guldberg, 2005; Donner et al., 2005). These studies based their conclusions on the assumption that the thermal threshold for a population of corals is relatively constant. It is important, therefore, that we understand the dynamics associated with the thermal threshold, especially when it comes to understanding how thermal history can influence bleaching response.

Reef-building corals are highly dependent on their symbiotic relationship with photosynthetic dinoflagellates (*Symbiodinium* sp.). As part of this mutualistic endosymbiosis, corals receive the majority of their carbon and energy requirements from *Symbiodinium* (Muscatine et al., 1984; Falkowski et al., 1993). The transfer of photosynthetic products from the *Symbiodinium* to the host enables corals to grow and calcify at high rates within the warm and sunlit subtropical and tropical waters (Barnes and Chalker, 1990). The loss of these symbiotic dinoflagellates due to environmental stresses (coral bleaching) is likely to impact the energy and carbon budget of corals, and may result in death if the stress is severe and prolonged (Glynn, 1996). Bleaching [disassociation of the endosymbiosis between coral and *Symbiodinium* (Hoegh-Guldberg and Smith, 1989)] results in reduced *Symbiodinium* cell densities and/or their photosynthetic pigments. The loss of *Symbiodinium* and/or pigments begins with the dysfunction of the photosynthetic apparatus of *Symbiodinium* (Iglesias-Prieto et al., 1992; Fitt and Warner, 1995; Warner et al., 1996; Iglesias-Prieto and Trench, 1997; Brown et al., 1999; Jones et al., 1998; Downs et al., 2000; Tchernov et al., 2004).

Organisms tend to have thermal tolerances that reflect the environment in which they are found. This can occur either through acclimation, where an organism alters its phenotype, or through adaptation, where propagules are better suited to altered conditions. Corals and their symbionts have adapted to geographical differences in sea temperature through genetic shifts in thermal tolerance over long periods of time (Coles et al., 1976; Hoegh-Guldberg, 1999), which ultimately defines the response of the coral holobiont to stress within a region (Donner et al., 2005). However organisms are also

able to shift their phenotypic responses to a limited extent through acclimation to environmental extremes (Schmidt-Nielsen, 1996). In this respect, many organisms show the ability to acclimate to stressors at both the physiological and molecular levels (Feder and Hofmann, 1999; Tomanek and Somero, 1999; Sorte and Hoffman, 2005). The coral holobiont is no exception, and several studies have demonstrated that corals (Brown et al., 2002; Coles and Brown, 2003; Castillo and Helmuth, 2005; Dove et al., 2006) and *Symbiodinium* (Iglesias-Prieto and Trench, 1997; Downs et al., 2000) can acclimate to heat and light stress. In addition, variations in the bleaching susceptibility of conspecifics across environmental gradients, for instance latitude, suggest the further potential for corals to acclimate to rising sea temperatures in the field (Coles and Brown, 2003; Donner et al., 2005; Ulstrup et al., 2006). The ability to acclimatize to different local conditions has the potential to play an important role in explaining small- and large-scale patterns in bleaching susceptibility.

The physiological behavior of reef-building corals is actively influenced by their dinoflagellate symbionts (Little et al., 2004; Berkelmans and van Oppen, 2006). Acclimation to light stress in *Symbiodinium* has been shown to occur through changes in peridinin chlorophyll *a*–binding protein complexes (PCP) and chlorophyll *a*–chlorophyll c_2–peridinin protein complexes (acpPCP), light harvesting pigments (Iglesias-Prieto and Trench, 1997), the efficiency of photosystem II (PS II) and xanthophyll turnover rates (Brown et al., 1999). *Symbiodinium* may acclimate to stressful conditions such as high light and temperature during periods of pre-exposure by the early activation of photo-protective mechanisms. Such mechanisms include changes in the efficiency of the xanthophyll cycle (Brown et al., 1999; Brown et al., 2000), photosynthetic efficiency (Anthony and Hoegh-Guldberg, 2003) and non-photochemical quenching.

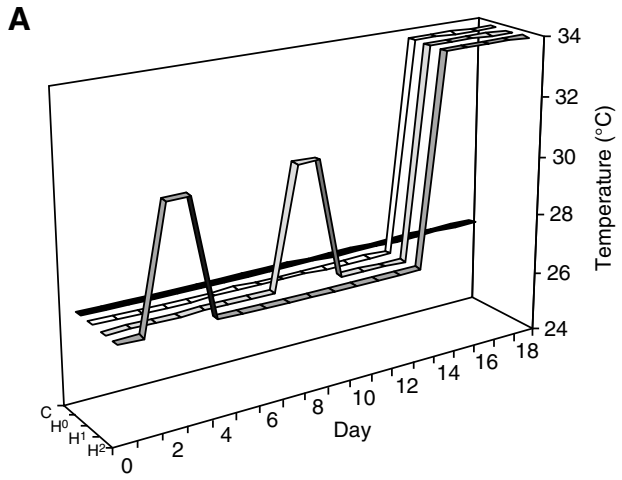
The ability of the coral symbiosis to acclimate to stressors has been ignored in much of the recent experimental work on coral bleaching that consists of single thermal stress events, which often do not mirror actual conditions. Corals located on reef crests are often exposed to thermal and light conditions above their predicted thresholds for several hours on consecutive days (R.A.M., personal observations). Few controlled experiments have attempted to determine the effect of short-term elevated temperatures on subsequent bleaching outcomes (Coles and Jokiel, 1978).

The aim of the present study was to explore the influence of thermal history on the response of the coral symbiosis (*Acropora aspera*) to thermal stress. In particular, our study explores the changes in photosystem II efficiency, non-photochemical quenching, xanthophyll cycling and symbiont density in response to thermal stress in pre-exposed (31°C) and control coral populations. In particular, the study investigates the effect of three different prior thermal stress histories on the acquisition of thermal tolerance in order to explore whether the pattern of pre-exposure is important over and above the actual amount of exposure.

MATERIALS AND METHODS

Collection and maintenance of corals

Branches of the reef building corals *Acropora aspera* [tan/cream morph (Dove, 2004)] containing *Symbiodinium* clade C3 (LaJeunesse et al., 2004) were collected from three large colonies on the reef flat adjacent to Heron Island Research Station (HIRS, 23°33′S, 151°54′E) in April 2006. Single upward-growing branch tips (3–4 cm long) were removed using wire cutters and transported to the seawater facility at HIRS and placed in racks immersed in running seawater. Coral branches were acclimated in 4 large



B

Experimental design

Treatment	H^2			H^1			H^0			Control		
Colony	1	2	3	1	2	3	1	2	3	1	2	3
Measurement	PS II HPLC Symbiont density			PS II HPLC Symbiont density			PS II HPLC Symbiont density			PS II HPLC Symbiont density		
Replication	Experiment replicated twice in two tank systems											

Fig. 1 (A) Thermal stress profiles simulated in the experiment using *Acropora aspera*. Experimental temperature regimes are shown for the three different treatments, H^2, H^1, H^0, and a control group (C). On day 2 (08:00 h), treatment H^2 was heated to 31°C for 48 h and then returned to ambient temperature. On day 8 (08:00 h), treatment H^1 was heated to 31°C for 48 h and then returned to ambient temperature. On day 14, H^2, H^1 and H^0 were heated to an average of 34°C for 5 consecutive days simulating a bleaching event. (B) The table shows the experimental design, which was replicated twice in two tank systems. PS II (photosystem II efficiency), HPLC (pigment analysis including xanthophyll and chlorophyll *a*) and *Symbiodinium* density were measured 3 times per treatment, per replicate.

experimental aquarium tanks to the mean local ambient temperature (27°C, drawn from the reef crest). Corals were exposed to natural reef flat summer daily light levels (average daily maximum 1271.28±40.07 μmol m^{-2} s^{-1}, average daily light dosage 21.37±0.88 mol m^{-2} day^{-1}) throughout the experiment.

Experimental treatments

The experimental system consisted of four 750 l tanks, two of which were heated treatment tanks and two of which were unheated controls. All coral branches were split evenly and placed in the control tanks at the beginning of the experiment. The experimental design involved pre-stressing corals 2 weeks and 1 week prior to a simulated bleaching event (Fig. 1A). To achieve this, 60 coral branches were moved from the control tanks to the treatment tank at 08:00 h 2 weeks and 1 week before the simulated bleaching event, whereupon the temperature was elevated to approximately 31°C over a 48 h period. After 2 days, corals were returned to the control tanks. Hereafter, those corals that were pre-stressed 2 weeks prior to the simulated bleaching event are called H^2, those pre-stressed 1 week prior are called H^1, and those that had not been pre-stressed H^0; in addition control corals were not subjected to the simulated bleaching event. To control for effects due to handling, all corals were moved

and handled whenever any corals were moved between tanks. Given that in total there were four different treatments, this experimental design was chosen in an effort to minimize inter-tank differences, which may have confounded comparison between treatments. Light levels were recorded using an ODYSSEY recorder (DATAFLOW Systems Pty Ltd, Christchurch, New Zealand) placed in each aquarium. Water temperature was recorded every 2 min using StowAway TidbiT Loggers placed in each aquarium (Onset Computer Corporation, Bourne, MA, USA).

Five coral branches per treatment were removed each day from the experiment at 18:00 h for measurement of photosynthetic efficiency. One branch per colony per tank for each corresponding treatment was snap-frozen using liquid nitrogen at 12:00 h on each day of treatment exposure and stored in a –70°C freezer. These branches were used to measure *Symbiodinium* cell densities, chlorophyll and xanthophyll pigment concentrations (Fig. 1B).

Measurements of photosynthetic efficiency

Effective dark-adapted quantum yield (F_v/F_m) is a relative measure of the rate at which PS II can use light to process electrons flowing during the photosynthesis and the photosynthetic efficiency of the light reactions (Hoegh-Guldberg and Jones, 1999). An imaging pulse amplitude modulation (PAM) chlorophyll fluorometer (MAXI Imaging PAM, Walz, Effeltrich, Germany) was used to analyse the photosynthetic efficiency of corals daily at 18:00 h. Five coral branches were measured per treatment per tank (totalling ten branches per treatment) in a glass Petri dish containing seawater from the flow-through system. Corals were dark-adapted for 40 min prior to measuring effective quantum yield (F_v/F_m) to assess whether PS II was adversely affected by the treatments (Warner et al., 1996).

Induction recovery curves were also performed to examine the ability of *Symbiodinium* to acclimate to short-term light stress. Coral branches were exposed to 461 μE m^{-2} s^{-1} for 6 min followed by a dark recovery period of 14 min. Photo-kinetic parameters, including non photochemical quenching and PS II quantum yield, were measured during the light period using a saturation pulse every second. A saturating pulse was used 16 times integrated over 13 min 58 s during the recovery phase. Dynamic yield and non-photochemical quenching were determined using the calculations of Warner et al. (Warner et al., 1996).

The extraction of non water-soluble pigment

Coral tissue from frozen coral branches was removed using an air brush and 5 ml of filtered (0.45 μm) seawater solution. *Symbiodinium* were separated from the coral tissue by centrifugation at 4500 ***g*** (4°C) for 5 min. The supernatant was discarded and the pellet resuspended in HPLC grade methanol (1 ml methanol for every 750 μl of sample). Solutions were filtered through a 0.22 μm membrane filter (GSWP04700, Millipore, North Ryde, NSW, Australia) and 50 μl sterilised milli-Q was then added to a 250 μl aliquots prior to use in High Performance Liquid Chromatography (HPLC). Samples were then separated and analysed using the methods of Dove et al. (Dove et al., 2006) and Zapata et al. (Zapata et al., 2000) using a SHIMADZU (Tokyo, Japan) SCL-10 HPLC attached to a SHIMADZU SPD-M10A photodiode array detector.

Cell densities of *Symbiodinium*

One branch per colony from each corresponding treatment within each tank was used to assess dinoflagellate density in *A. aspera*. Density was determined using a SEDGEWICK rafter cell 550 (ProSciTech S8050, Kirwin, Queensland, Australia). Fourteen 1 μl cells were counted within a 1 ml slide, and averaged per sample.

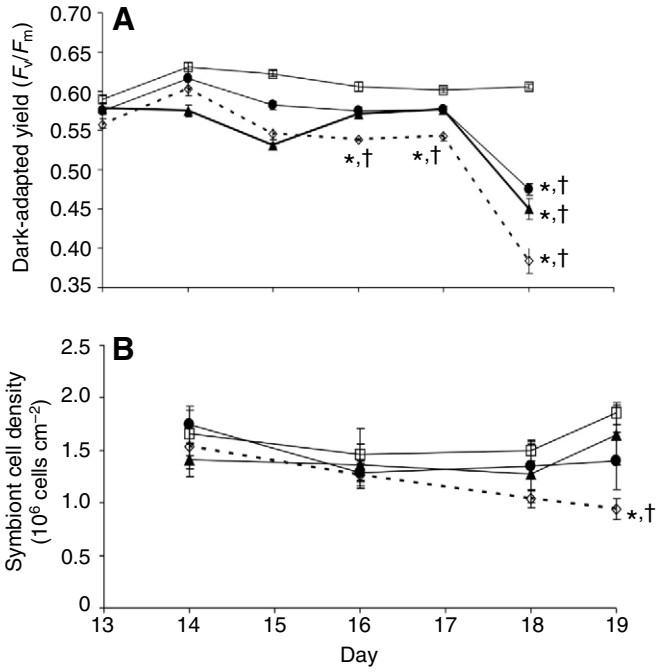


Fig. 2. Changes in the (A) dark-adapted quantum yield (18:00 h) and (B) *Symbiodinium* cell density within the coral *Acropora aspera* during a simulated bleaching event. Coral colonies preheated 2 weeks prior (H^2; filled circles), 1 week prior (H^1; filled triangles), not preheated (H^0; open diamonds), and controls (open squares) are indicated. Error bars, although not always visible, represent ± s.e.m., N=10. *Significant difference (*post-hoc* LSD analysis, P<0.05) between treatment and control on the same day; †significant difference (*post-hoc* LSD, P<0.05) between treatments on the same day.

Coral surface area was determined using the melted paraffin technique (Stimson and Kinzie, 1991).

Statistical analysis

Trends within the data stemming from the measurement of dark-adapted PS II fluorescence yield, non-photochemical quenching, xanthophyll ratios, chlorophyll *a* and *Symbiodinium* density data were analysed using a multivariate repeated-measures ANOVA with STATISTICA 7.0 (Statsoft Inc., Tulsa, OK, USA). Tank, time and preheat treatment were treated as within factors. Following a significant 'Preheat×Time' interaction and a non significant 'Preheat×Tank' interaction, differences were determined between treatments using Fisher's Least Significant Difference (LSD) test for *post-hoc* comparisons. Summaries of ANOVA statistics are shown in supplementary material Tables S1–S5; both significant and non-significant statistics are reported in the text.

RESULTS

The effect of prior thermal stress on the efficiency of *Symbiodinium* Photosystem II apparatus and *Symbiodinium* density

The photosynthetic response of *Symbiodinium* varied significantly between the three thermal treatments and was significantly different to the controls (Fig. 2A). There were significant effects of day, treatment and day and treatment interactions ($F_{12,200}$=8.81, P<0.001) on the dark-adapted PS II yield in *Symbiodinium*. *A. aspera* branches that were not preheated experienced the largest decline in efficiency of PS II by comparison to treatments that were preheated 2 and 1

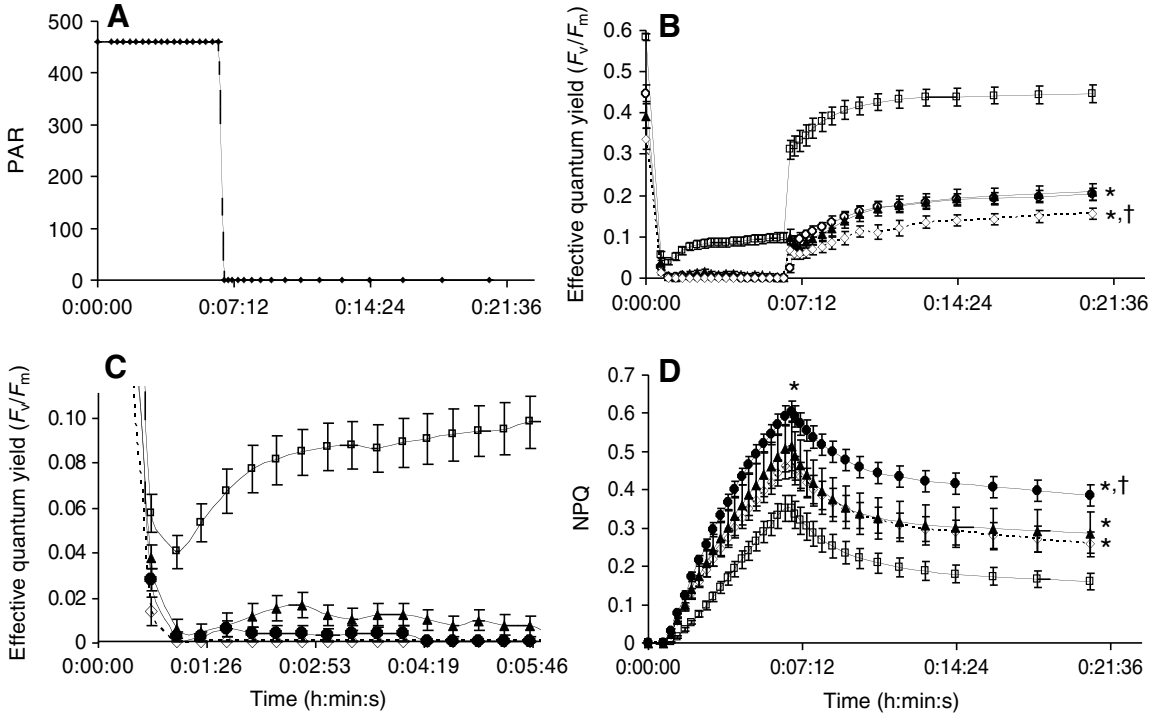


Fig. 3 Light induction recovery curves for effective quantum yield of photosystem II and NPQ for *Symbiodinium* within colonies of the reef-building coral *Acropora aspera* recorded at 18:00 h. (A) Amount of photosynthetically active radiation (PAR) that the coral branches were exposed to throughout the light induction recovery curve. (B) Effective quantum yield measurements for day 18. (C) The actinic light period showing the gradual saturation of photosystem II. (D) NPQ measurements for day 18. Corals preheated 2 weeks prior- (H^2; filled circles), 1 week prior (H^1; filled triangles), and not preheated (H^0: open diamonds) were then subjected to a simulated bleaching event (control; open squares). Error bars shown, but not always visible, represent ± s.e.m., N=10. *Significant difference (*post-hoc* LSD analysis, P<0.05) between treatment and control on the same day; †significant difference (*post-hoc*, LSD, P<0.05) between treatments on the same day.

weeks prior to the main experimental period of thermal stress (days 16, 17 and 18, P<0.001) (Fig. 2A). Corals that were preheated 2 and 1 weeks prior also experienced a significant decline, but this was significantly less during the last three time points, indicating reduced damage to the efficiency of the PS II reaction centre.

The population density of *Symbiodinium* (cells cm^{-2}) in those corals that had not been pre-stressed also showed a significant decrease at the final sampling point (day 19) as compared to treatments that did not undergo prior thermal stress (P=0.005, Fig. 2B). The population density of dinoflagellates in corals preheated 2 weeks and 1 week prior remained relatively constant across the experiment, ranging between 1.3 and 1.7×10^6 cells cm^{-2}, and was not significantly different from unheated controls.

Induction recovery curves were performed to indicate the speed of recovery of the PS II reaction centre from PAR pressure (Fig. 3A) and thermal stress. Induction recovery curves performed on corals during the final evening (day 18) of the experiment revealed that the recovery potential of those preheated 1 and 2 weeks prior to the prolonged thermal stress was greater than those not preheated (Fig. 3B, P=0.02). After 6 min of light exposure (461 μmol quanta m^{-2} s^{-1}, 400–700 nm), the PS II reaction centre in corals that did not undergo prior thermal stress became saturated almost immediately (Fig. 3C). This effect was significantly less in corals that were preheated 2 weeks prior and those preheated 1 week prior to the main experimental period of thermal stress ($F_{54,216}$=13.44, P<0.001).

Non-photochemical quenching (NPQ) data obtained from the induction recovery curve from day 18 (Fig. 3D) provides a measure of how intact PS II is on the cessation of the simulated bleaching event. Dark-adapted NPQ measured over the duration of the prolonged thermal stress period is shown in Fig. 4A. An increase in rate of dark-adapted NPQ on Day 18 can be correlated with a pronounced decline in dark-adapted fluorescence yield in all samples that had been heated, due to its role in the dissipation of excess heat, in response to pressure on PS II. A higher rate of NPQ in treatment corals compared to the control can be seen in the light induction recovery curve recorded on day 18 (Fig. 3D) showing a significant effect of treatment (P=0.029). Corals preheated 2 weeks prior to the bleaching event had significantly higher NPQ values than those preheated 1 week prior and those not preheated during both actinic illumination (P=0.00075) and dark acclimation period (P=0.01). The induction recovery curve recorded 2 days prior (data not shown) saw corals preheated 1 week prior having a significantly lower rate of NPQ in comparison to the two other treatments at the end of the acclimation period (P=0.023). Therefore, it can be inferred that during the last 3 days of the simulated bleaching event (days 16–18), there is an increase in rate of NPQ in coral branches preheated 2 weeks prior, a decrease in rate of NPQ in corals not preheated, and no difference in rate of NPQ in corals preheated 1 week prior.

The effect of prior thermal stress treatments on the composition of photosynthetic pigments in *Symbiodinium* sp.

No significant interaction between 'Time×Treatment' was found for the effects on Chl *a* $cell^{-1}$ (Fig. 4B $F_{9,96}$=1.45 P=0.24). There was, however, a significant interaction between prior treatment and

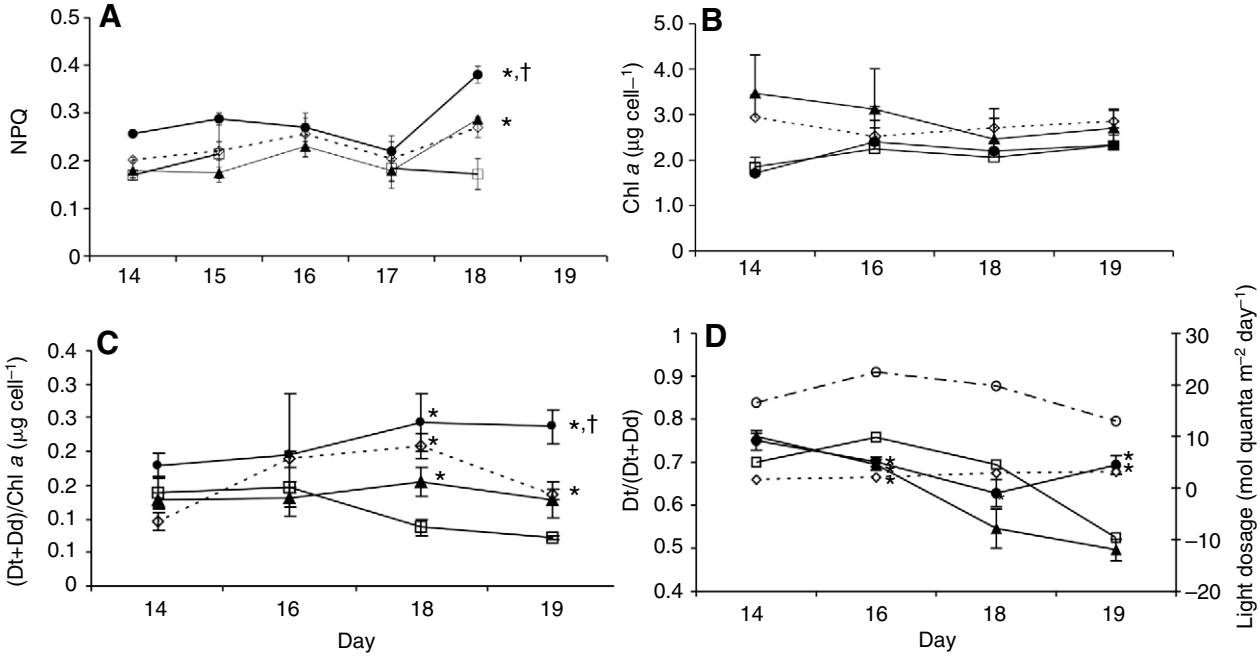


Fig. 4. Changes in (A) dark-adapted NPQ, control from day 16 missing due to Imaging Pam malfunction, (B) chlorophyll *a*, (C) xanthophyll pool size and (D) daily light dosage (open circles) and xanthophylls cycling in *Symbiodinium* within the tissues of the coral *Acropora aspera* during a simulated bleaching event. Coral colonies preheated 2 weeks prior (H^2; filled circles), 1 week prior (H^1; filled triangles), and not preheated (H^0; open diamonds) and controls (open squares) are indicated. Error bars shown, but not always visible, represent ± s.e.m., *N*=10. *Significant difference (*post-hoc* LSD analysis, *P*<0.05) between treatment and control on the same day; †significant difference (*post-hoc* LSD, *P*<0.05) between treatments on the same day. *N*=10 for A, *N*=6 for B,C.

time for the ratio of the xanthophyll pool to Chl *a* ($F_{9,96}$=2.6, *P*=0.04) (Fig. 4C). Corals preheated 2 weeks prior showed a gradual increase in concentration of diatoxanthin (Dt) and diadinoxanthin (Dd) to Chl *a* throughout the main experimental period of thermal stress from day 14 to 19 (Fig. 4C). Day 19 shows a significant decrease in the ratio of Dd and Dt to Chl *a* in corals not preheated (*P*=0.044), whereas there is no significant change from day 18 to 19 in the other two treatments. Diadinoxanthin becomes de-epoxidated to form diatoxanthin in dinoflagellates (Ambarsari et al., 1997; Brown et al., 1999) and performs a critical role in dissipating excess energy from the antenna of PS II. *Symbiodinium* in the corals preheated 2 weeks prior and in those not preheated had greater xanthophyll pool size than those preheated 1 week prior and the control (Fig. 4C, Table 1). A significant increase in diatoxanthin was seen in corals that were not exposed to prior thermal stress conditions (day 19, *P*=0.007), contributing significantly to the increased pool size, which was twofold greater that in corals preheated 1 week prior.

A significant interaction was found between 'Time×Treatment' in the xanthophyll cycling ratio (Dt)/(Dt+Dd) ($F_{9,96}$=13.5, *P*<0.001), with the greatest difference between treatments occurring on day 19 (Fig. 4D). Day 19, the final day of the experiment where the decline in effective quantum yield of PS II was greatest the previous day, saw a significant increase in xanthophyll cycling in corals preheated 2 weeks prior and corals not preheated (*P*<0.0001 in both), whereas there was no significant difference in dinoflagellates from corals preheated 1 week prior to the bleaching stress event.

Table 1. Summary of results from physiological parameters measured in *Acropora aspera* taken from three colonies on Heron Island reef crest

Parameter	Heated 2 weeks prior	Heated 1 week prior	Not pre-heated
Effective yield of PS II (F_v/F_m)	↓	↓	⇊
NPQ	↑↑	↑	↑
Symbiodinium density	–	–	↓
Xanthophyll pool (Dt+Dd)	↑	–	↑
Xanthophyll pool/Chl *a*	↑	–	↓
Xanthophyll cycling Dt/(Dt+Dd)	↑	–	↑

Corals were heat stressed for either 2 weeks, 1 week or not pre-stressed prior to the main experimental period, averaging 34°C. Effective PSII yield, NPQ, *Symbiodinium* densities and xanthophyll ratios are displayed in greater detail in the figures. Arrows indicate significant difference (↑, increased; ↓, decreased) from control on the final day of the simulated bleaching event. Double arrows indicate significant difference between treatments.

Dt, diatoxanthin; Dd, diadinoxanthin.

DISCUSSION

Coral reefs have shown large-scale and rapid changes in response to climate change. In this respect, small increases in temperature have been found to have profound effects on the endosymbiotic relationship between reef-building corals and their dinoflagellates (Hoegh-Guldberg, 1999; Hughes et al., 2003). A large number of studies have demonstrated that corals and their symbionts are living close to their current thermal maxima. Projections that combine the behaviour of corals with projections of sea temperature from global climate change models suggest that mass bleaching events will become a yearly phenomenon within the next 30–50 years (Hoegh-Guldberg, 1999). This is expected to lead to a rapid decline in coral cover on tropical reef systems, which may be the basis of the observed decline seen by a number of recent studies (e.g.

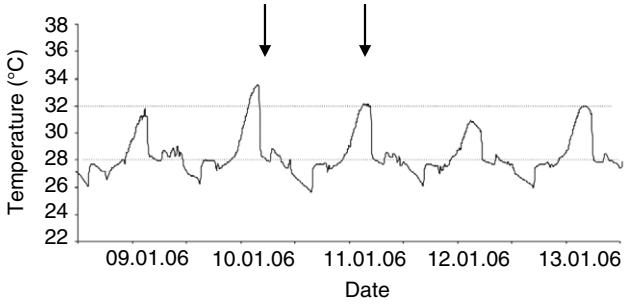


Fig. 5. Sea surface temperature recorded on Heron Island reef crest (HIRS, 23°33′S, 151°54′E) from the 9th to the 13th January 2006. Arrows indicate logger data where SST exceeded 32°C on two consecutive days for periods over 4 h.

Bruno and Selig, 2007). Given that these models depend heavily on the behaviour of the thermal threshold, it is important that variability stemming from acclimation is understood. This study is one of a small number that has focused on experimentally exploring the effect of issues such as thermal history on the tendency of reef-building corals and their dinoflagellate symbionts to undergo bleaching.

Several studies have recently highlighted the importance of understanding the flexibility of coral responses to temperature and other environmental stresses (Coles and Brown, 2003). Hoegh-Guldberg (Hoegh-Guldberg, 2000) and Donner et al. (Donner et al., 2005) estimated that the thermal tolerance of reef-building corals will have to rise by approximately 0.2–1.0°C per decade to keep pace with rising global temperatures. Thermal history has also been shown to lead to acclimation in coral conspecifics at intra-colony (e.g. Brown et al., 2002), colony, reefal (Castillo and Helmuth, 2005) and latitudinal (Hoegh-Guldberg, 1999; Ulstrup et al., 2006) scales. However, only a single study has examined thermal acclimation in corals experimentally (Coles and Jokiel, 1978). Given that coral bleaching is predominantly associated with short-term increases in temperature, ranging from a few hours to a few days, and that these bleaching conditions may be preceded by warmer than average temperatures (Fig. 5), we examined if short-term prior thermal stress leads to increased thermal tolerance through acclimation.

Corals that were subjected to warmer than average temperatures prior to thermal stress beyond the bleaching threshold were found to be more thermally tolerant, as indicated by reduced loss of *Symbiodinium* compared to corals that had not been pre-stressed (Fig. 2B). While pre-stressed coral symbiont densities were unchanged at the end of the bleaching, symbiont densities in non-prestressed corals declined by approximately 40%. Given that the *Symbiodinium* photosynthetic apparatus has been suggested as the point of thermal lesion in a number of studies (Iglesias-Prieto et al., 1993; Warner et al., 1996; Jones and Hoegh-Guldberg, 1999), we explored the responses by corals and their symbionts with respect to photosynthetic efficiency and pigment profiles, particularly the xanthophyll pool, which provides the majority of NPQ.

The dark-adapted yield of photosystem II has been used extensively as a measure of damage to photosystem II and has been shown to be a conventionally good proxy for bleaching susceptibility (Warner et al., 1999; Jones et al., 2000; Fitt et al., 2001). Those corals that had not been stressed had significantly lower dark-adapted yields than pre-stressed and control corals in the final 3 days of the bleaching event (Fig. 2A), indicating that pre-stress provided some level of thermal protection to photosystem II. Significant differences between the yield of control and pre-stressed corals were only seen on the final day of the experiment. In the case of corals that were pre-stressed 2 weeks prior to bleaching (H^2), tolerance was correlated with higher levels of NPQ (Fig. 2D) and xanthophyll pool to Chl *a* ratio (Fig. 4B) while the xanthophyll cycling remained unchanged relative to non-prestressed controls (Fig. 4C).

A very different pattern was observed in xanthophyll cycling for those corals pre-stressed 1 week prior to bleaching. Despite bleaching less than non-prestressed corals, these pre-stressed corals did not differ from control corals with respect to xanthophyll pool size (Table 1) and cycling rate (Fig. 4C). The ratio of diatoxanthin to dinoxanthin and diatoxanthin was significantly less than those seen in coral that were pre-stressed 2 weeks prior and those not pre-stressed (Fig. 4C). The rate of NPQ was also found to be less in corals prestressed 1 week prior compare to other prestressed corals (Fig. 3D). These contrasting patterns of NPQ, xanthophyll levels and xanthophyll cycling indicate that acclimation is a dynamic process, with distinct differences in stress profiles that differed by 7 days. In addition it indicates that a variety of other thermal protective pathways are occurring in the coral holobiont in addition to those examined here. This is not surprising given that acclimation in the holobiont can involve the coral host, *Symbiodinium*, or a combination of both (Dove, 2004). Both corals (Kortschak et al., 2003) and *Symbiodinium* (Leggat et al., 2007) have been shown to possess a wide variety of genes that encode for stress response proteins (e.g. heat shock proteins, superoxide dismutase, ubiquitin etc.), which can impart protection, indicating that a more comprehensive study is required to elucidate all of the underlying mechanisms of thermal acclimation.

Although commonly interchanged, the terms acclimation and heat shock refer to very different cellular responses (Bowler, 2005). Acclimation results from long-term exposure, in the order of days to weeks, to new conditions that are within the normal limits of an organism's response. Acclimation generally results in a variety of cellular and molecular responses such as alteration of lipid composition, protein isozymes and protein expression, which will provide protection from long-term gradual changes in the environment. In contrast heat shock generally refers to a period of short exposure to near lethal temperature that may or may not provide a very distinct cellular and molecular response to those seen in acclimation. Which cellular and biochemical mechanisms occur in the coral holobiont to different conditions will be a future area of important research. An examination of the literature on perhaps the most well-studied invertebrate model for acclimation, *Drosophila* sp., demonstrates that the ability of an organism to acclimate to various conditions, how it acclimates and, indeed, the costs of acclimation, can vary significantly between con-specifics and species and have profound effects on an organism (for a review, see Hoffman et al., 2003). For example, the induction of acclimatory responses has been shown to have effects on a variety of other performance measures resulting in such disparate responses as increased longevity, increased cold tolerance and decreased fertility, to list a few (Hoffman et al., 2003). A history of exposure to a range of stresses has also been found to alter subsequent higher plant responses where a priming stress activates genes that leave an epigenetic mark, facilitating a quicker response to subsequent stresses (Bruce et al., 2007). What costs and benefits accrue to the coral holobiont will need to be determined.

This study conclusively demonstrates that thermal stress events 2 weeks and 1 week prior to a bleaching event provide significantly increased thermal tolerance to the coral holobiont, suggesting that short time-scale thermal adaptation can have profound effects on coral bleaching. The inclusion of physiological and acclimatory properties into the modeling of climate change on populations and

ecosystems has already been advocated (Helmuth et al., 2005); in the case of the coral holobiont, a better understanding of thermal history on bleaching susceptibility may provide insights into what reefs will look like in the near future under altered climate regimes.

LIST OF ABBREVIATIONS

acpPCP	chl *a*–chl c_2–peridinin protein complex
Chl *a*	chlorophyll *a*
Dd	diadinoxanthin
Dt	diatoxanthin
NPQ	non-photochemical quenching
PAM	pulse amplitude modulation
PAR	photosynthetically active radiation
PCP	peridinin chl *a*–binding protein complex
PS II	photosystem II

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Table S1 Repeated measures ANOVA results for PAM fluorescence yield (dark adapted F_v/F_m) across five time points with time, preheat and tank as within factors

source	SS	d.f.	MS	*F*	sig.
preheat	0.230	3	0.077	54.9	**<0.001**
preheat X time	0.134	12	0.011	8.81	**<0.001**
preheat X tank	0.001	3	0.000	0.34	0.798
preheat X time X tank	0.014	12	0.001	1.11	0.378
error (preheat)	0.017	12			

Table S2. Repeated measures ANOVA results for xanthophyll ratios [Dt/(Dt+Dd)] across four time points with time, preheat and tank as within factors

source	SS	d.f.	MS	*F*	sig.
preheat	0.046	3	0.015	5.7	**0.034**
preheat X time	0.320	9	0.036	13.5	**<0.001**
preheat X tank	0.012	3	0.004	1.4	0.338
preheat X time X tank	0.076	9	0.009	1.6	0.182
error (preheat)	0.016	6	0.003		

Table S3 Repeated measures ANOVA results for xanthophyll pool size (Dt+Dd) across four time points with time, preheat and tank as within factors

source	SS	d.f.	MS	*F*	sig.
preheat	0.046	3	0.015	5.7	**0.035**
preheat X time	0.320	9	0.036	13.5	**<0.001**
preheat X tank	0.012	3	0.004	1.4	0.338
preheat X time X tank	0.076	9	0.008	1.6	0.182
error (preheat)	0.016	6	0.003		

Table S4 Repeated measures ANOVA results for xanthophyll pool to Chl *a* ratio [(Dt+Dd)/chl *a*] across four time points with time, preheat and tank as within factors

source	SS	d.f.	MS	*F*	sig.
preheat	0.082	3	0.028	6.8	**0.023**
preheat X time	0.086	9	0.010	3.3	**0.016**
preheat X tank	0.033	3	0.011	2.8	0.127
preheat X time X tank	0.063	9	0.007	1.2	0.36
error (preheat)	0.024	6	0.004		