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SHORT NOTE

Decadal environmental 'memory' in a reef coral?

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Abstract West sides of the coral *Coelastrea aspera*, which had achieved thermo-tolerance after previous experience of high solar irradiance in the field, were rotated through 180° on a reef flat in Phuket, Thailand (7°50′N, 98°25.5′E), in 2000 in a manipulation experiment and secured in this position. In 2010, elevated sea temperatures caused extreme bleaching in these corals, with former west sides of colonies (now facing east) retaining four times higher symbiont densities than the east sides of control colonies, which had not been rotated and which had been subject to a lower irradiance environment than west sides throughout their lifetime. The reduced bleaching susceptibility of the former west sides in 2010, compared to handling controls, suggests that the rotated corals had retained a 'memory' of their previous high irradiance history despite living under lower irradiance for 10 years. Such long-term retention of an environmental 'memory' raises important questions about the acclimatisation potential of reef corals in a changing climate and the mechanisms by which it is achieved.

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Introduction

Variability in bleaching patterns within individual coral colonies, subject to elevated temperatures, has been well documented in the field. Such patterns have been attributed to spatial distributions of resident symbiotic algal clades (Rowan et al. 1997), localised high irradiance stress falling on upper coral surfaces (Fitt et al. 1993) and experience-mediated physiological tolerances (Brown et al. 2002a).

In the latter example, western sides of the merulinid (Huang et al. 2014) coral *Coelastrea* (formerly *Goniastrea*) *aspera*, which had received regular high solar irradiance, showed less bleaching and improved temperature tolerance compared with more shaded eastern sides during a major temperature-induced bleaching event in the Andaman Sea in 1995 (Brown et al. 2000). *C. aspera* from inshore reef locations, where this phenomenon was observed, hosts only one *Symbiodinium* clade, namely D1a (Pettay and LaJeunesse 2009), and so, bleaching patterns cannot be attributed to genetic differences in symbiotic algae as described in other studies (Rowan et al. 1997). This superior temperature tolerance was attributed to higher levels of stress proteins and antioxidants in the coral host and improved xanthophyll cycling in *Symbiodinium* on western surfaces (Brown et al. 2002a). The acquisition of thermal tolerance by western sides of *C. aspera* colonies led to striking bleaching patterns in the field in 1995, where eastern surfaces of many *C. aspera* colonies bleached stark white, while western sides were still highly pigmented. The

majority of these colonies were estimated to be 4–6 years old, and the timescale of acquisition of thermal tolerance would therefore have been relatively short (2–3 years) since colonies do not reach a size sufficient to receive differential solar irradiance regimes on east and west surfaces until they are at least 2 years of age (Brown et al. 1994).

An earlier review (Brown and Cossins 2011) first raised the question of how long the irradiance 'memory', and the thermo-tolerance it conferred in these corals, might be retained in the absence of the environmental signal that first induced the tolerance. A manipulation experiment in 2000 where C. *aspera* colonies were rotated through 180° so that original west surfaces faced east and east surfaces faced west (McDougall et al. 2006), together with an extreme bleaching event in 2010 (Brown and Phongsuwan 2012), provided an opportunity to assess the bleaching susceptibility of former western surfaces that had been orientated in a more shaded east-facing direction for 10 years.

Materials and methods

Study site and coral manipulation

The study site is an intertidal reef flat at Ao Tan Khen on the SE tip of Phuket, Thailand (7°50′N, 98°25.5′E), which has been a focus of study for over 35 years and which has been described in detail in earlier work (reviewed in Brown et al. 2011). In November 2000, a manipulation experiment was carried out on the inner reef flat, which is dominated by the massive corals *C. aspera* and *Porites lutea* (McDougall et al. 2006). *C. aspera* colonies ($n = 24$), approximately 20 cm mean diameter, were carefully detached from the reef using a hammer and stone chisel and rotated 180° before being cemented in their new position (hereafter termed 'rotated'). An additional 24 colonies were detached from the reef and replaced in their original position to act as handling controls (hereafter termed 'controls') (Fig. 1a). Earlier detailed levelling of the reef flat (Tudhope and Scoffin 1994) ensured that colonies were all placed at a similar height on the reef and repeated observations through ebbing and flooding tides confirmed that both 'rotated' and 'control' colonies were exposed and covered by seawater at the same time.

Environmental data

A long-term record of monthly mean sea temperatures for the region was obtained from the UK Meteorological Office Hadley Centre (HadSST2). These values have been shown to reliably track '*in situ*' thermistors at the site which have been in place for varying time periods since 1994 (Dunne 2012).

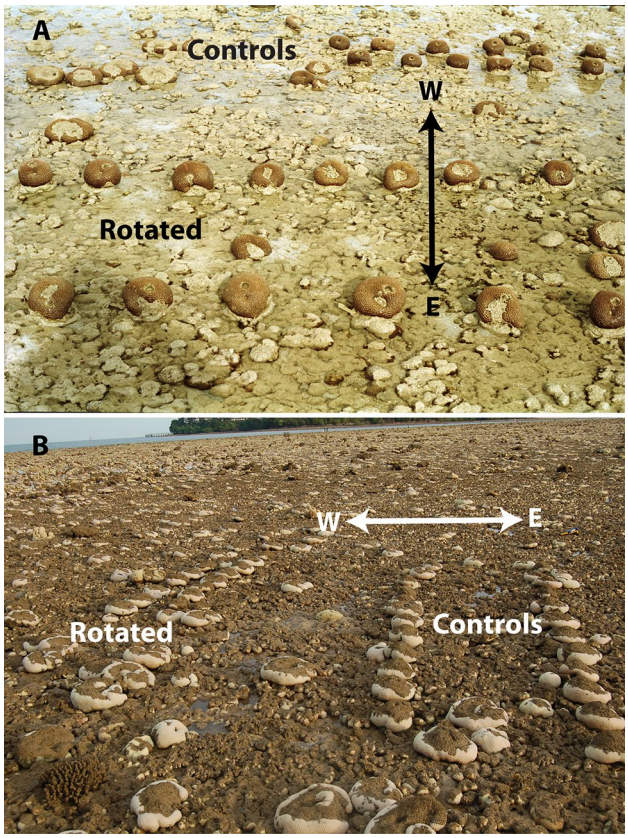

Fig. 1 Location of 'rotated' and 'control' corals on the reef flat (**a**) in November 2000 (**b**) in May 2010 during the extreme bleaching event

Coral sampling and measurement of *Symbiodinium* densities

At the height of a severe bleaching event in June 2010, two hole punch samples (1.5 cm diametre) were collected from the east face of each of five colonies from 'rotated' and 'control' groups. One hole punch sample was used to estimate *Symbiodinium* density, while the other was used for genetic analysis of the *Symbiodinium* clade diversity.

Symbiodinium density was measured in fixed and decalcified coral samples using the standard methodology described in Brown et al. (1999) where algae were counted in homogenised tissues on haemocytometer slides and values expressed on a surface area basis. Samples for algal genetic determination were preserved in molecular grade ethanol stored at −20 °C and transported to the UK for analysis.

Genetic analysis of *Symbiodinium*

DNA was extracted from all samples using Qiagen DNeasy Blood and Tissue kits. Variation in *Symbiodinium* was monitored using the ITS2 region, using the forward primer

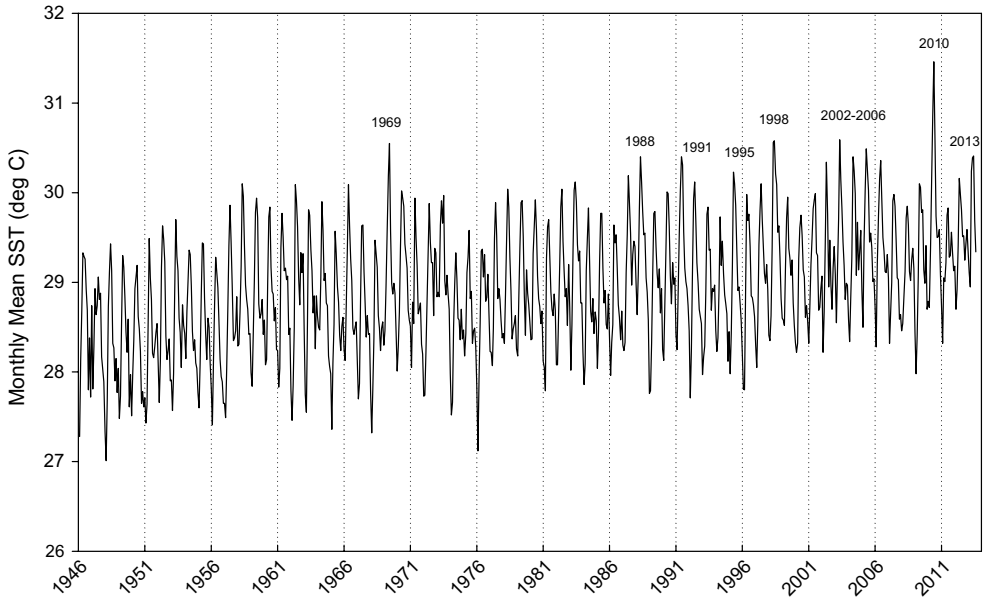

Fig. 2 Monthly mean sea temperatures (1946–2013) for the sea area around Phuket, Thailand, from the HadSST 2 dataset. Years are highlighted when sea temperatures rose above 30.1 °C

'ITSintfor2' and the highly conserved reverse primer that anneals to the LSU 'ITS2CLAMP' (LaJeunesse et al. 2003). PCR mixture and protocol were the same as those described in Sweet (2013). Denaturing gradient gel electrophoresis (DGGE) was performed using the D-Code universal mutation detection system (Bio-Rad). PCR products were resolved on 10 % (w/v) polyacrylamide gels containing a 30–60 % denaturant gradient for 13 h at 60 °C and a constant voltage of 50 V. Gels were stained with 9 µl Sybr Gold (Sigma) in 50 µl of TAE for 20 min and then washed in 500 ml 1X TAE for a further 30 min and visualised using a UV transilluminator. A subset of the dominant resulting bands was excised from DGGE gels, left overnight in Zigma Molecular grade H_20, vacuum centrifuged, re-amplified with the ITS2 primers and sequenced using a Big Dye transformation sequence kit and sent to Genevision (Newcastle University, UK) for sequencing. *Symbiodinium* sequences were defined from DGGE band-matching analysis using Bionumerics 3.5 (Applied Maths BVBA). Tolerance and optimisation for band matching were set at 1 %.

Statistical analysis

The hypothesis to be tested was that *Symbiodinium* densities in 'rotated' colonies would be greater than in 'control' colonies. Data were normal and homoscedastic, which would permit the use of the parametric one-tailed *t* test. However, to obtain a better estimate of the *p* value, a one-tailed exact permutation test (using the Package permTS; Fay and Shaw 2010) was run in R (R Core Team 2013).

An analysis of similarity (ANOSIM) was conducted to test differences in the OUT patterns associated with *Symbiodinium* ITS gene assemblage.

Results and discussion

In May 2010, sea temperatures at the study site rose to unprecedented high levels (Fig. 2), causing a major bleaching event during which all manipulated corals bleached (Fig. 1b). Previous major coral bleaching events at the site were recorded only in 1991 and 1995 (Brown and Phongsuwan 2004) although higher temperatures were seen in other years (Fig. 2). Annual photography of permanent phototransects at the site showed only partial bleaching in *C. aspera* colonies on the reef flat in May 1991 and 1995 and 100 % bleaching in May 2010 with no thermally induced bleaching recorded in any other year (Brown unpubl.).

Symbiodinium densities in east sides of bleached 'rotated' colonies (i.e. former west surfaces) were significantly higher compared with those in east sides of bleached 'controls' in 2010 ($p = 0.0119$, one-tailed exact permutation test) (Fig. 3). East sides of 'rotated' corals harboured four times the number of *Symbiodinium* (mean $= 0.792 \times 10^7$ cm^{-2}) compared to 'controls' (mean $= 0.17 \times 10^7$ cm^{-2}). Genetic analysis of the *Symbiodinium* clades showed no significant difference (ANOSIM $R = 0.87$, $p = 0.167$) between the clades present in control or rotated corals and confirmed earlier work (LaJeunesse et al. 2010), which showed that inner reef flat *G. aspera*

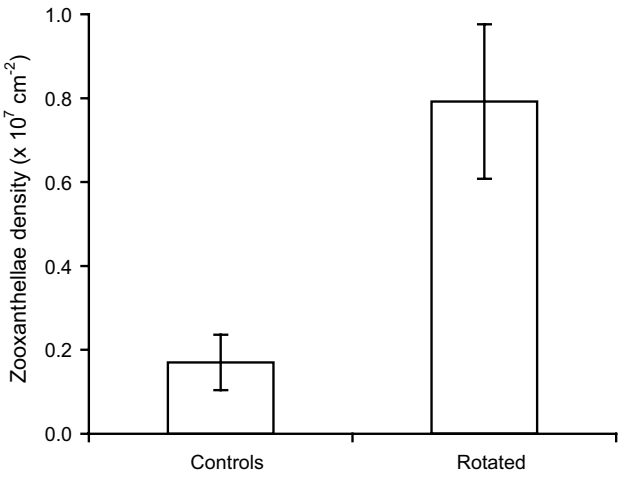

Fig. 3 Mean zooxanthellae densities ($\times 10^7 cm^{-2}$) ($\pm$SE) in 'rotated' and 'control' bleached corals

colonies harbour only one algal clade that of D1a (Unique GenBank accession no. for this study; KP001551).

Such results suggest that the thermal tolerance conferred by previous experience of high solar irradiance in *C. aspera* can be retained for at least 10 years despite the former west surfaces having been exposed to a lower irradiance environment during this period. The fact that former irradiance experience can shape the susceptibility of a coral to bleaching has been well established (Brown et al. 2002b; Brown and Dunne 2008). However, the question of how long this environmental 'memory' might last under reduced irradiance was unknown.

The persistence of such a 'memory', over a 10-year period, raises interesting questions about the possible mechanism(s) underlying this observation. It is well known that exposure of other organisms to environmental stressors can modulate the genome resulting in changed epigenomes and transcription profiles (Turner 2009). As a result, the genome bears epigenetic marks, particularly methylation and hydroxymethylation of CpG sites that determine, in part, how the genome responds through regulation of transcription (Bird 2002; Holliday 2005). The subject of epigenetics has been raised in discussion in a number of papers relating to coral thermal acclimatisation (Middlebrook et al. 2008; Brown and Cossins 2011; Bellantuono et al. 2012; Palumbi et al. 2014), yet so far firm evidence for an epigenetic basis to coral acclimatisation has been lacking. In the present study, samples were taken for analysis of methylated DNA, but unfortunately all were lost in a malfunctioning freezer.

Nevertheless, the apparent existence of a long-term environmental 'memory' in the coral *C. aspera* is worth recording since it is clear from previous work that in the early development of west surfaces of these colonies, aspects of their stress physiology are significantly modified (Brown et al. 2002a). The mechanisms underlying the long-term persistence of a reduced bleaching susceptibility in a reduced irradiance environment remain to be elucidated, but the implications for future acclimatisation potential of corals in a changing climate are considerable. Future work to address the existence of a 'memory' in this coral species would investigate not only DNA methylation but also gene expression in east and west sides subject to an experimental temperature elevation.

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