Outbreak and persistence of opportunistic symbiotic dinoflagellates during the 2005 Caribbean mass coral ‘bleaching’ event

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Reef corals are sentinels for the adverse effects of rapid global warming on the planet's ecosystems. Warming sea surface temperatures have led to frequent episodes of bleaching and mortality among corals that depend on endosymbiotic micro-algae (*Symbiodinium*) for their survival. However, our understanding of the ecological and evolutionary response of corals to episodes of thermal stress remains inadequate. For the first time, we describe how the symbioses of major reef-building species in the Caribbean respond to severe thermal stress before, during and after a severe bleaching event. Evidence suggests that background populations of *Symbiodinium trenchi* (D1a) increased in prevalence and abundance, especially among corals that exhibited high sensitivity to stress. Contrary to previous hypotheses, which posit that a change in symbiont occurs subsequent to bleaching, *S. trenchi* increased in the weeks leading up to and during the bleaching episode and disproportionately dominated colonies that did not bleach. During the bleaching event, approximately 20 per cent of colonies surveyed harboured this symbiont at high densities (calculated at less than 1.0% only months before bleaching began). However, competitive displacement by homologous symbionts significantly reduced *S. trenchi*'s prevalence and dominance among colonies after a 2-year period following the bleaching event. While the extended duration of thermal stress in 2005 provided an ecological opportunity for a rare host-generalist symbiont, it remains unclear to what extent the rise and fall of *S. trenchi* was of ecological benefit or whether its increased prevalence was an indicator of weakening coral health.

Keywords: Caribbean; climate change; competitive displacement; coral bleaching; opportunistic species; *Symbiodinium*

1. INTRODUCTION

Rapid climate change is negatively impacting many of the planet's ecosystems through the disruption of numerous important ecological processes (Walther *et al.* 2002). In particular, coral reefs exhibit extreme sensitivity to episodes of prolonged thermal stress (reviewed in Fitt *et al.* 2001; Coles & Brown 2003); temperatures of just a few degrees above mean summer highs can cause bleaching involving significant losses of mutualistic symbiotic dinoflagellates (micro-algae) from the tissues of corals (Porter *et al.* 1989; Jones & Yellowlees 1997). Such episodes of mass bleaching and mortality adversely affect the health, growth and fitness of coral communities throughout the world (Fitt *et al.* 2001; Coles & Brown 2003; McClanahan *et al.* 2009). Therefore, given the predicted rise in sea surface temperatures (SSTs), the functional integrities of coral reef ecosystems are threatened in even the most pristine regions (Hoegh-Guldberg *et al.* 2007; IPCC 2007).

The phylogenetic incongruence between host and symbiont phylogenies indicates that partnerships do

change over evolutionary time scales (Rowan & Powers 1991; LaJeunesse 2005). The coevolution of coral–algal symbioses, therefore, appears to involve periodic changes in host–symbiont specificity expressed by numerous host taxa (LaJeunesse 2005). Indeed, global phylogeographic patterns suggest that evolutionarily divergent lineages of *Symbiodinium* (i.e. clades) have attained ecological dominance and undergone adaptive radiations coinciding with changes in long-standing environmental conditions (Rowan & Powers 1991; LaJeunesse 2005) lasting many thousands or even millions of years (LaJeunesse 2005). For this reason, there is considerable uncertainty about whether coral symbioses can respond to the current trends in SST warming over shorter ecological time scales (Buddemeier & Fautin 1993; Donner *et al.* 2005; Baird *et al.* 2007).

It has been proposed that severe coral bleaching may expedite changes among coral populations such that more individuals will harbour thermally tolerant symbionts (Buddemeier & Fautin 1993; Baker 2001; Baker *et al.* 2004). A precipitous loss of the native resident symbiont (i.e. during bleaching) may be necessary to facilitate the re-population of a colony via ‘shuffling’ (advantageous growth of a background resident population) or, possibly, by ‘switching’ (uptake of a new symbiont from

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Electronic supplementary material is available at <http://dx.doi.org/10.1098/rspb.2009.1405> or via <http://rspb.royalsocietypublishing.org>.

the environment) to a species of symbiont that is more tolerant of thermal and/or high light stress (Rowan *et al.* 1997; Baker 2001; Berkelmans & van Oppen 2006). It also has been suggested that the rapid acquisition of a stress-tolerant symbiont will either lessen, or delay, the impact of rapid global warming on coral communities and their ecosystems (Baker 2001; Berkelmans & van Oppen 2006; Jones *et al.* 2008). However, the possibility for rapid change to occur in coral–algal symbioses through ‘shuffling’ and/or ‘switching’ during bleaching is challenged by the fact that many individual hosts appear to maintain highly specific, homogeneous and stable associations (Goulet 2006; Thornhill *et al.* 2006a,b; Sampayo *et al.* 2008; Stat *et al.* 2009; Thornhill *et al.* 2009).

There is surprisingly little information about the influence of a major thermal disturbance on host–symbiont specificity; although recent biogeographical surveys have documented high frequencies of *Symbiodinium* in clade D in certain areas affected by widespread bleaching (Baker *et al.* 2004; but see Oliver & Palumbi 2009). The ideal way to assess whether bleaching can meaningfully influence the ecology of these mutualisms is to directly track how symbiont populations in a variety of corals change or remain stable over the duration of a natural bleaching event. Yet, rarely has the identity of a symbiont in bleached and unbleached colonies been established and tracked before, during and after an episode of mass coral bleaching (during: Glynn *et al.* 2001; LaJeunesse *et al.* 2007; Goulet *et al.* 2008; before and soon after: Jones *et al.* 2008; Sampayo *et al.* 2008; Stat *et al.* 2009; Thornhill *et al.* 2009). Of the few studies characterizing partner specificity and/or change following an episode of thermal stress and bleaching, most focused on a single coral genus or species and few have tracked these associations for more than a year following recovery.

A rare opportunity presented itself during the 2005 Caribbean mass coral bleaching event to evaluate the stability and specificity of various coral–algal symbioses exposed to severe stress. We used ITS-denaturing gradient gel electrophoresis (DGGE) fingerprinting and real-time PCR (rtPCR) to track the prevalence and intra-colony abundance of *Symbiodinium trenchi* nom. nud (*D1a sensu* LaJeunesse 2002; provisionally named in LaJeunesse *et al.* 2005), a known stress tolerant and potentially opportunistic species (Toller *et al.* 2001a,b; Thornhill *et al.* 2006b) in coral communities around the island of Barbados before and during the bleaching event, and then six months and two years later.

2. MATERIAL AND METHODS

(a) Sea surface temperatures

Mesoscale (50 km grid) SST data for Barbados (14°00′00″ N and 60°00′00″ W) were obtained through the NOAA/NESDIS Coral Reef Watch website (<http://coralreefwatch.noaa.gov/index.html>). Values for each month were averaged for three consecutive years prior to 2005 (January 2001 to December 2003) and graphed against temperatures recorded for 2005.

(b) Bleaching surveys at Atlantis bank reef

Beginning in October 2005, immediately following the rapid onset of widespread bleaching, the coral communities on Atlantis bank reef (more than 15 m) and Bachelor’s Hall

fringing reef (less than 5 m), situated off the west (leeward) coast of the island of Barbados (figure 1a), were surveyed to quantify the extent and severity of bleaching. Five 20 metre long, 1 metre wide belt transects were laid out on each reef and the total number of ‘healthy’ (i.e. live, unbleached) colonies and live bleached colonies were recorded. Bleaching is defined in this study as any coral colony showing ‘loss of coloration’ (i.e. abnormal/bleached pigmentation). Most bleached colonies were totally white in October and, therefore, no index of partial bleaching was recorded. These reefs were resurveyed in February 2006 and again in June 2006 to determine the extent of coral mortality and/or recovery. For details on how colony health and mortality were calculated, see Oxenford *et al.* (2008a,b).

(c) Sample collections

Small fragments from numerous host taxa were acquired using a hammer and chisel and placed into individual plastic bags. Collections were made in mid-July and early August 2005 from Atlantis bank reef (more than 15 m) and Bachelor’s Hall fringing reef (less than 5 m) (figure 1a). Colonies sampled during this time appeared healthy and displayed no ‘visual’ signs of stress-induced bleaching. Atlantis bank reef and Bachelor’s Hall fringing reef were resampled in early December 2005 when collections were made from the top surfaces of bleached and normally pigmented colonies from eight common scleractinians (see §3). Collections from these species were made again in late April and early May 2006 and in November 2007.

(d) DNA extractions, ITS-DGGE analysis and sequencing

Samples were processed to separate symbionts from host tissues as previously described (LaJeunesse *et al.* 2003). The resulting algal pellet was preserved in a solution of 20 per cent DMSO, 0.25 M EDTA, and NaCl-saturated water (Seutin *et al.* 1991). Nucleic acids were extracted using the Wizard DNA preparation protocol (Promega, Madison, WI, USA) as modified by LaJeunesse *et al.* (2003).

For each DNA extract, the ITS 2 region was amplified using primers ‘ITS 2 clamp’ and ‘ITSintfor 2’ (LaJeunesse & Trench 2000) with the touch-down thermal cycle given in LaJeunesse (2002). Products from these PCR reactions were electrophoresed for 15 h at 115 V on denaturing gradient gels (45–80%) using a CBScientific system (Del Mar, CA, USA). Gels stained with Sybergreen (Molecular Probes, Eugene, OR, USA) were photographed.

The identification of old and new symbiont PCR–DGGE fingerprint signatures was verified by excising brightly stained bands from the denaturing gel. The DNA was eluted in 500 µl H₂O, re-amplified using the same primer set without the GC-rich clamp in a standard PCR thermal cycle profile (annealing set at 52°C for 40 cycles) and directly sequenced using ABI Prism Big Dye 3.1 Terminator Cycle Sequencing reagents with 3.2 pmol of the reverse ITS primer (without the GC clamp). Reaction products were analysed on an Applied Biosystems 3100 Genetic Analyzer (Division of Perkin Elmer, Foster City, CA, USA).

(e) ITS-DGGE detection limits for *Symbiodinium trenchi* (D1a)

Artificial combinations from cells of freshly isolated *S. trenchi* (*D1a sensu* LaJeunesse 2002) and *Symbiodinium* C3-e were created to test the sensitivity of ITS-DGGE for detecting background populations (LaJeunesse 2002;

Figure 1. (a) Collection sites on the island of Barbados in the eastern Caribbean. Populations of various coral taxa were repeatedly sampled from the deep (more than 15 m) Atlantis bank reef site and the shallow (less than 5 m) Bachelor's Hall fringing reef site before, during, six months following and two years following the 2005 bleaching event. (b) Average monthly SSTs around Barbados between January 2001 and December 2003 (error bars mean $\pm$ s.d. based on the average of monthly means) compared with SSTs in 2005 (open circle). Arrows indicate times of sampling during 2005. All values calculated from 50 km global satellite data at approximate coordinates 14°00'00" N and 60°00'00" W (readings taken every 48 h; error bars mean $\pm$ s.d.).

Thornhill *et al.* 2006b; LaJeunesse *et al.* 2008). Freshly isolated *S. trenchi* from *Montastraea faveolata* and C3-e from *Montastraea cavernosa* originating from Key West, Florida, were used as sources of symbiont material. Cell densities were determined from five replicate cell counts using a hemocytometer. Appropriate volumes containing known numbers of isolated cells were added to each other to produce cell ratios of each symbiont that ranged from about 2 per cent to approximately 99 per cent. The DNA was then extracted from these mixtures and analysed as described above.

(f) Real-time PCR

Having sampled prior to the onset of bleaching provided the opportunity to determine whether *S. trenchi*, described as

opportunistic (Toller *et al.* 2001a,b; Thornhill *et al.* 2006b), was already present at low abundance in many colonies before the start of bleaching, or was subsequently introduced during or immediately following the event. While rtPCR is inherently quantitative, the data in this study were evaluated qualitatively to provide a more sensitive (when compared with ITS-DGGE) assessment of the presence or absence of Clade D *Symbiodinium*.

Background populations of *S. trenchi* were determined using an rtPCR protocol developed by Ulstrup & van Oppen (2003) and modified by Smith (2008). All amplification reactions were run on an MJ research qPCR thermal cycler with CDF-3240 Chromo4 detection system (BioRAD) using TaqMan chemistry (Applied Biosystems). Fluorescence data were collected and analysed using MJ OPTICON MONITOR analysis software v. 3.1 (BioRAD). Universal cycling parameters for quantitative TaqMan assays were followed (Applied Biosystems): 2 min at 50°C, 10 min at 95°C, 40 cycles of 15 s at 95°C and 1 min at 60°C.

The rtPCR cycle threshold or $C(t)$ value limit for *S. trenchi* detection was determined previously by evaluating a series of cultured *Symbiodinium* controls (Smith 2008). A common fluorescence threshold of 0.017 was used for all runs analysed to permit comparison of $C(t)$ values across separate runs (Livak & Schmittgen 2001). The $C(t)$ value of 33.0 was set as the upper limit for 'positive' and was one complete cycle below the lowest $C(t)$ observed in the negative controls, thereby reducing the likelihood of Type I error and providing a definitive estimate of *S. trenchi* background populations.

For rtPCR analysis of field-collected samples, standard curves were run on each 96-well reaction plate along with no-template negative controls to assess any non-specific fluorescence signal. Triplicate rtPCR runs were performed for each sample. Standard curves were constructed using genomic DNA isolated from isoclonal *S. trenchi* cultures. Sample and culture DNA were quantified spectrophotometrically and normalized to 1.0 ng μl^{-1} using MilliQ H₂O. Standard curves were generated as a series of five (1.0–0.0001 ng μl^{-1}) 10-fold serial dilutions and plotted as $C(t)$ versus the logarithm of the corresponding concentration. The OPTICON software calculated a linear regression line through the standard curve data points and both the slope and r^2 -value were reported. $C(t)$ values for unknown samples were generated by comparison with the calibration curve(s) within the same run (i.e. same 96-well plate). A sample was scored positive for *S. trenchi* if the $C(t)$ values were 33.00 or less in two out of three independent reactions.

(g) Statistical analysis of presence absence of *Symbiodinium trenchi*

The frequencies of *S. trenchi* detected using ITS-DGGE fingerprinting and/or rtPCR in samples from before, during and after the bleaching event were analysed using Pearson's χ^2 -test. The null hypothesis was that the frequency distribution of *S. trenchi* would be similar across all sampling times.

3. RESULTS

(a) Sea surface temperatures, coral bleaching, recovery and mortality

In comparison with monthly averages of three combined years, January 2001 to December 2003, monthly SSTs for 2005 were 0.5–2.0°C above normal throughout much of the year before bleaching began in September

(figure 1b). Wide variances in bleaching were observed among common species of reef-building coral. Populations of *Montastraea annularis* complex and *Siderastrea siderea* contained the highest proportion of bleached individuals (figure 2a,b) and exhibited the highest percentage of mortality (figure 2c). Many colonies from both species were slowest to recover, requiring eight months or more for normal pigmentation to return (figure 2b).

(b) ITS-DGGE and rtPCR detection limits for *Symbiodinium trenchi*

During analyses of cell mixtures with *S. trenchi* and *Symbiodinium* C3-e, ITS-DGGE fingerprinting detected the presence of C3-e at proportions as low as 1 per cent, but detected *S. trenchi* only when it existed in proportions greater than 20 per cent (electronic supplementary material, figure S3a). In contrast, the rtPCR protocol detected cell concentrations of *S. trenchi* as low as 0.1 per cent of the total symbiont population (electronic supplementary material, figure S3b; Smith 2008). The genomes of clade C *Symbiodinium* have a ribosomal copy number that is three to five times greater than clade D and is therefore easier to detect in mixed samples (Smith 2008).

(c) The host distribution, frequency and dominance of *Symbiodinium trenchi* before, during and months after the 2005 bleaching event

Among the eight coral species surveyed throughout the study, only *M. cavernosa* lacked detectable levels of *S. trenchi* before the bleaching event while colonies of *S. siderea* exhibited the highest frequency of occurrence and intra-colony abundance of *S. trenchi* (figure 4). In addition to hosting *S. trenchi* before the bleaching event, *Symbiodinium* C7-a was found in all deep (more than 15 m) colonies of *M. annularis*, while type B1j was also found in shallow dwelling colonies, often present in mixtures with C7-a (less than 5 m; electronic supplementary material, figure S1a).

The proportion of colonies harbouring *S. trenchi* at background and high densities increased in about half of the coral taxa surveyed during (December 2005) and following (April and May 2006) the bleaching event (figure 4). Furthermore, many unbleached colonies of *M. annularis*, *S. siderea*, *Agaricia* spp. and *M. cavernosa* sampled during the bleaching event were dominated by *S. trenchi* (e.g. electronic supplementary material, figures S1 and S2). *Symbiodinium trenchi* was also detected in many bleached colonies of these same species (see below). The proportion of *Meandrina meandrites* colonies with background levels of *S. trenchi* increased significantly (figure 4), but never reached high enough abundances to be detected by ITS-DGGE (electronic supplementary material, figure S2c). The proportion of colonies in the genus *Porites* with detectable levels of *S. trenchi* did not change appreciably throughout the study's duration. Only one colony of *Porites astreoides* in May 2006 harboured high densities of *S. trenchi* detectable by ITS-DGGE.

The analysis of bleached and unbleached *M. annularis* colonies from early December 2005 (during the bleaching event) found that *S. trenchi* was the dominant symbiont in all darkly pigmented colonies sampled ($n = 7$, electronic

Figure 2. (a) Condition of coral communities in Barbados in late October during the bleaching event of 2005. (b) The differential bleaching and (c) mortality were assessed among five common coral species on Atlantis bank reef. Percentages of colonies bleached are based on belt transect surveys conducted during the greatest extent of bleaching (0 = October 2005), four months later (4 = February 2006) and again eight months (8 = June 2006) after temperatures returned to normal levels. (c) Coral mortality shown as percentages of tissue loss averaged across colonies of each species at four and eight months after the height of bleaching.

supplementary material, figure S1b). A wide variety of symbionts including C7-a, *S. trenchi*, and B1 were observed in bleached colonies ($n = 7$). In four of seven samples taken from bleached colonies, an unusual clade A species, designated A13 (formerly A1.1, *sensu* LaJeunesse 2001 and tentatively named *Symbiodinium cariborum*, cf. LaJeunesse 2001), was identified (electronic supplementary material, figure S1b).

During the bleaching event, *S. trenchi* was detected in all colonies of *S. siderea* at either high or low relative abundances (in bleached and unbleached colonies; figure 3 and electronic supplementary material, figure S2a). Some colonies with normal pigmentation also hosted populations of *Symbiodinium* C3 and B5 (electronic supplementary material, figure S2a), indicating that there was no absolute correspondence with bleaching and the identity of the dominant resident symbiont for this host.

Figure 3. Percentages of colonies representing eight coral species where *S. trenchi* was detected as a dominant symbiont (black shade, 20–100%; electronic supplementary material, figure S3a) via ITS-DGGE fingerprinting and/or at low abundance background levels (grey shade, 0.1–20%; electronic supplementary material, figure S3b) using rtPCR, before (July/August 2005), during (December 2005 and April/May 2006) and 2 years after bleaching (November 2007). Not all taxa were resurveyed in November 2007. An asterisk by the coral species name indicates statistically significant differences (Pearson's χ^2 -test) in frequencies of *S. trenchi* among different sampling times. The numbers of individual coral colonies sampled for each species in each time period are indicated.

By late April and early May 2006, most surviving colonies of *M. annularis* were brown in colour. The relative frequency and dominance of *S. trenchi* among colonies sampled was high and relatively unchanged in this host since December 2005 (figure 4a). *Symbiodinium B1j* was again common among the shallow colonies at Bachelor's Hall; however, *C7-a* was not detected in any of these shallow colonies (electronic supplementary material, figure S1c).

(d) Presence of *Symbiodinium trenchi* after a two-year recovery

With the exception of one colony of *Diploria labyrinthiformis*, analyses of *Diploria* spp., *Agaricia* spp. and *M. meandrites* sampled in November 2007 found no trace of *S. trenchi* using ITS-DGGE and rtPCR (figure 3). In contrast, *S. trenchi* remained in at least some colonies of *M. annularis* and *S. siderea*, although its prevalence and abundance in *M. annularis* were significantly lower than during and months following the bleaching event (figure 4a).

At the shallow Bachelor's Hall site, no *S. trenchi* were detected in *M. annularis* by ITS-DGGE or rtPCR; instead the tops and sides of eight out of nine colonies were dominated exclusively by *B1j* (figure 4b) and one colony appeared homogeneous for *B1*. At the deep water Atlantis reef bank site, *Symbiodinium C7-a* was harboured exclusively by 11 (68%) *M. annularis* colonies.

ITS-DGGE identified high abundances of *S. trenchi* in five (32%) colonies with *Symbiodinium C7-a* also present in over half of these individuals. *Symbiodinium trenchi* was not detected at background concentrations in any of the colonies (figure 4b).

For *S. siderea* sampled in 2007, *S. trenchi* dominated all seven colonies sampled at the shallow Bachelor's Hall site, but was only found in three of nine colonies at the deep Atlantis site and only at background levels (figure 4b). *Symbiodinium C3* was the dominant symbiont in all nine of the deep colonies and two of these also contained high abundances of *Symbiodinium B5* (detected using ITS-DGGE).

4. DISCUSSION

The short-term and long-term ecological responses of coral–micro-algal symbioses to severe thermal stress are more complex than currently realized. The patterns observed during the 2005 Caribbean bleaching event indicate that environmental stressors may facilitate ecological opportunities for rare, background and/or heterologous *Symbiodinium* by disrupting the homeostasis between host and symbiont. This further suggests that the coral host may have little influence over whether or not

Figure 4. (a) Change in the prevalence (frequency of occurrence among individual colonies) and abundance (grey, background; black, dominant) of *S. trenchi* in the population of *M. annularis* between July/August 2005 and November 2007. The prevalence and abundance of this *Symbiodinium* are presumed to have increased leading up to the onset of bleaching (October 2005) and remained high into April/May 2006, but then dropped precipitously by November 2007. Different lower case letters above bars denote statistically significant differences (χ^2 , $p < 0.01$) in the frequency of *S. trenchi* among time periods. The numbers of colonies sampled are given in parentheses. (b) Frequency and abundance of *S. trenchi* in two dominant reef-building corals by habitat depth in November 2007 showing patterns of loss and/or persistence of *S. trenchi*. At the shallow Bachelor's Hall site, colonies of *M. annularis* were dominated by host specific *B1j*, while colonies of *S. siderea* contained *S. trenchi* exclusively. No differences in symbionts were observed on the tops and sides of each shallow colony of *M. annularis*. In contrast, at the deep Atlantis site, *S. trenchi* was abundant in some colonies of *M. annularis*, but was detected only at background levels in *S. siderea*. Numbers of individual colonies sampled are provided in parentheses.

a particular symbiont proliferates in its tissues under stressful circumstances.

(a) *Symbiodinium trenchi* as an opportunistic symbiont

Gathering of ecological data on *S. trenchi* has been difficult because surveys of reef cnidarian assemblages from shallow and deep habitats throughout the greater Caribbean rarely detect this symbiont (Baker & Rowan 1997; LaJeunesse 2002; Thornhill *et al.* 2006a,b; Warner *et al.* 2006; Smith 2008; but see Kemp *et al.* 2006). *Symbiodinium trenchi* (clade E *sensu* Toller *et al.* 2001a,b, reassigned to clade D, LaJeunesse 2001; Baker 2001) was first described in the western Caribbean where it

was harboured by colonies of *M. annularis* persisting in marginal reef habitats characterized by low water flow, periodic episodes of high turbidity and/or wide temperature fluctuations (Toller *et al.* 2001a,b; LaJeunesse 2002; Garren *et al.* 2006). Further details of *S. trenchi*'s ecology emerged when it was identified in some colonies recovering from experimentally induced bleaching (Baker 2001; Toller *et al.* 2001b). However, its long-term persistence was never assessed (Toller *et al.* 2001b) until several colonies of *M. annularis* in the Florida Keys, USA, harbouring *S. trenchi* were monitored seasonally for seven years after initially bleaching in 1998. By the end of 2002, *S. trenchi* was no longer detectable as the dominant symbiont population in these colonies. This was the first indication that populations of *S. trenchi* can be displaced by homologous symbionts under normal environmental conditions (Thornhill *et al.* 2006b). The observation that *S. trenchi* occurs in colonies growing under harsh environmental conditions, but is unstable relative to other *Symbiodinium* when hospitable conditions return, suggests that it is primarily opportunistic (Toller *et al.* 2001a,b; Thornhill *et al.* 2006b).

Opportunistic species are defined by particular ecological/physiological traits that make them competitive in marginal habitats (Pianka 1970). Situated in the far eastern Caribbean, Barbados lacks many of the lagoonal coral communities where *S. trenchi* would be expected to thrive (Toller *et al.* 2001a; Garren *et al.* 2006). This may have changed in 2005 when temperatures were 1–2°C higher than normal for most of the year in the eastern Caribbean (figure 1b). The community-wide survey conducted across all reef habitats in Barbados during July/August 2005 comprising 285 cnidarian hosts from 34 genera found a higher than normal proportion of coral colonies with abundant levels of *S. trenchi* (5 versus 1–2% in other regions of the Caribbean; Smith 2008) and suggests that these symbioses were experiencing stress by mid-summer, months before the onset of visual bleaching. The ecological significance of a higher than usual prevalence of *S. trenchi* was not fully appreciated until rtPCR analyses identified unusually high frequencies of *S. trenchi* at background levels in Barbadian corals, relative to those in the Gulf of Mexico, Belize, St Croix and the Bahamas sampled in previous non-bleaching years (22 versus an average of 5%, Smith 2008). The high prevalence of background populations of *S. trenchi* in July/August appears to have transitioned to widespread dominance across numerous colonies of many coral species during the coral bleaching that ensued in September and October and into the early phases of recovery in April/May 2006 (figure 3). The highest frequencies and abundances of *S. trenchi* were found in coral species whose populations exhibited the greatest sensitivity to thermal stress (compare figure 2b,c with figure 3) and suggests a relationship exists between a colony's state of stress and the presence of this symbiont.

The prevalence and intra-colony abundance of *S. trenchi* increased significantly from July/August 2005 to December 2005 (from less than 1.0% to approx. 20%; $p < 0.01$). Many colonies of *Agaricia* spp., *M. annularis* and *M. cavernosa* that remained unbleached harboured *S. trenchi* in high abundance (figure 4a and electronic supplementary material, figure S1b). We infer that this transition from background levels to high abundances of *S. trenchi* in colonies of various coral species

occurred mostly in the weeks preceding and during the bleaching event. Background resident populations of *S. trenchi* may have benefited competitively from the thermal stress that began before July/August and lasted into October (figure 1b). A calculation of population growth rates *in hospite* indicates that under the right circumstances, displacement by *S. trenchi* through direct or indirect competition would require only a few months (Jones & Yellowlees 1997). This assumes that growing populations of *S. trenchi* tolerated the thermal stress while normal, or homologous, symbionts ceased to grow and/or were differentially expelled. Using previously published estimates of cell division rates (e.g. 5 and 10 days; Wilkerson *et al.* 1988) and growing from starting concentrations of 1000 and 5000 cells cm⁻¹ (the limit range for detection using rtPCR; electronic supplementary material S3b), analyses show that *S. trenchi* would reach densities above 1 million cm⁻² within 1.5–3 months (electronic supplementary material, figure S4) and would be enough to make a colony appear brown and healthy during the height of bleaching (Fitt *et al.* 2000).

Marked changes in the prevalence and dominance of *S. trenchi* during the bleaching event indicate that symbiont ‘shuffling’ can and does occur without bleaching (see Thornhill *et al.* 2006b). Therefore, the destabilizing effect of stress alone may facilitate displacements, perhaps through direct or indirect competition, by opportunistic *Symbiodinium* among colonies of various host species and offers a general explanation for how extreme disturbances may influence certain coral–algal partnerships.

The prevalence and abundance of *S. trenchi* may have increased further during the initial phases of recovery. *Symbiodinium trenchi* was detected in most bleached colonies of *M. annularis* (approx. 80%; figure 4a) and may have proliferated advantageously during the months when host tissues were re-browning (Toller *et al.* 2001b). Indeed, five to six months after the peak of bleaching, the proportion of *M. annularis* colonies dominated by *S. trenchi* reached its maximum (68%; figure 4a and electronic supplementary material, figure S1c). The impressive increase in frequency and inter-colony dominance of this symbiont in these corals during and after this episode of thermal stress and bleaching suggests that, under certain conditions, a symbiont can spread rapidly through a host population.

Obvious questions arise about the physiological health of colonies whose symbiont populations have been displaced, or replaced, by *S. trenchi*. While the presence of this *Symbiodinium* may increase tolerances to high temperatures, what are the long-term consequences, if any, to the coral’s fitness and growth? Future analysis of the quality and quantity of translocated nutrients to the host, annual growth rates and reproductive capacities among colonies of various species dominated by *S. trenchi* relative to colonies harbouring homologous symbiont species would further resolve the nature of this stress-induced symbiotic interaction.

(b) The persistence and instability of *Symbiodinium trenchi* populations

Populations of *S. trenchi* may not remain stable in the years following the return of normal environmental conditions (Thornhill *et al.* 2006b). *Symbiodinium trenchi* was virtually absent in most host taxa resurveyed in

November 2007 and exhibited a marked reduction in *M. annularis*, especially in shallow habitats (figures 3 and 4a). Additionally, *Symbiodinium* C7-a, common among shallow *M. annularis* before the event, were also absent (electronic supplementary material, figure S1a). By comparison, one-third of deep-dwelling *M. annularis* colonies still harboured abundant densities of *S. trenchi*, but often in combination with C7-a (figure 4b). These depth differences in the frequency and abundance of *S. trenchi* in *M. annularis* indicate that displacement of an opportunist proceeds at different rates in different colonies and is dependent on light and/or the competitive ability of the homologous symbiont. A summary schematic reconstructing how the thermal stress of 2005 differentially influenced shallow and deep communities of *M. annularis* colonies before, during and after the bleaching event is presented in figure 5.

The long-term stability and persistence of *S. trenchi* is probably dependent on both intrinsic and extrinsic factors. The differences in the patterns of recovery and change among shallow and deep colonies of *S. siderea* and *M. annularis* emphasize the importance of the host species and depth in governing the displacement or persistence of this heterologous symbiont (figure 4b). *Symbiodinium trenchi* appears to be a poor competitor when environmental conditions are stable and may explain why *S. trenchi* occurs rarely in coral communities living in hospitable (i.e. mild) Caribbean reef environments (Toller *et al.* 2001a; LaJeunesse 2002; Thornhill *et al.* 2006a,b; Warner *et al.* 2006). Little is known about this species’s physiological capability to acclimate to environmental stress. Possessing higher tolerances to wide ranges in environmental conditions would explain its ecological limitations (i.e.) when competing under normal conditions with *Symbiodinium* that are more physiologically specialized (ecological versus physiological trade-offs, see McNaughton & Wolf 1970).

(c) Concluding remarks

Increasing atmospheric and oceanic temperatures are destabilizing many of the world’s ecosystems (Walther *et al.* 2002). The studied effects of climate change on animal–microbe interactions have been concerned mostly with the spread of pathogenic or toxic species (Harvell *et al.* 2004), but what of the influence of rising SSTs on the distribution and biology of ‘mutualistic’ microbes in the marine environment? Intensifying episodes of extreme thermal stress may facilitate the opportunistic rise of unusual host-generalist *Symbiodinium* spp., such as *S. trenchi*. While *S. trenchi*’s prevalence and abundance diminishes under normal environmental conditions, as the warming of SSTs continues, this opportunist may become increasingly more common and persistent, especially among corals whose natural symbionts have a greater sensitivity to thermal stress.

Sustaining the viability of coral reef ecosystems is important to the future economic and social well-being of many human communities. The ecological response of *S. trenchi* populations to the 2005 Caribbean mass bleaching could be interpreted optimistically because their increase in abundance may have ‘saved’ some colonies from bleaching and death. Conversely, the emergence of this species could be the byproduct of severe

Figure 5. A reconstruction of how thermal stress influenced *Symbiodinium* populations among shallow and deep colonies of *M. annularis* (numbered 1–8) inferred from molecular ecological surveys before, during and after the 2005 Caribbean bleaching event. Initially, colonies harboured high-light (*B1j*, yellow dots) and/or low-light distributed (*C7-a*, blue dots) *Symbiodinium* that are specific to *M. annularis*. *Symbiodinium trenchi* (red dots) occurred in some of these colonies, but at low and variable abundances. As the thermal stress worsened, *S. trenchi* increased in frequency and dominance, presumably through higher rates of growth so that by the height of bleaching, many bleached and unbleached colonies sampled at both depths contained relatively high concentrations of *S. trenchi*. Additional opportunistic symbionts such as *A13* (black dots) appeared in severely bleached colonies undergoing partial or total mortality. The frequency of occurrence and abundance of *S. trenchi* remained high months into recovery. *Symbiodinium trenchi* would later be competitively displaced over time, but more rapidly in colonies exposed to higher irradiance. Years later, *Symbiodinium C7-a* has not returned to shallow colonies of *M. annularis*, which now mostly contain homogeneous populations of *Symbiodinium B1j*.

stress and, therefore, an indicator for weakening coral health. More information is required about the ecology and physiology of *S. trenchi* in order to gauge the importance of this symbiont to the host community and ecosystem as a whole. Overall, the observations reported here indicate that responses of coral–algal symbioses to climate change are dynamic and complex and that gaps in knowledge limit accurate predictions about the future of coral reef ecosystems in a time of global warming.

We thank Renata Goodridge for assistance in the field. Two anonymous reviewers provided comments that improved the quality of the manuscript. A Coral Reef Research Permit to TCL and HAO was obtained through the CZMU, Government of Barbados. This research was supported by the National Science Foundation (IOB 544854 to T.C.L.), Florida International University, Pennsylvania State University and the University of the West Indies.

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