PROCEEDINGS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Resistance to thermal stress in corals without changes in symbiont composition

Anthony J. Bellantuono, Ove Hoegh-Guldberg and Mauricio Rodriguez-Lanetty

Proc. R. Soc. B published online 5 October 2011
doi: 10.1098/rspb.2011.1780

PROCEEDINGS OF THE ROYAL SOCIETY B

FirstCite® e-publishing

Proc. R. Soc. B
doi:10.1098/rspb.2011.1780
Published online

Resistance to thermal stress in corals without changes in symbiont composition

Anthony J. Bellantuono[1], Ove Hoegh-Guldberg[2,3] and Mauricio Rodriguez-Lanetty[1,*]

[1]*Department of Biology, University of Louisiana at Lafayette, Lafayette, LA 70504, USA*
[2]*Global Change Institute, University of Queensland, Saint Lucia, Queensland 4072, Australia*
[3]*Australian Research Council Centre of Excellence for Coral Reef Studies, James Cook University, Townsville, Queensland 4811, Australia*

Discovering how corals can adjust their thermal sensitivity in the context of global climate change is important in understanding the long-term persistence of coral reefs. In this study, we showed that short-term preconditioning to higher temperatures, 3°C below the experimentally determined bleaching threshold, for a period of 10 days provides thermal tolerance for the symbiosis stability between the scleractinian coral, *Acropora millepora* and *Symbiodinium*. Based on genotypic analysis, our results indicate that the acclimatization of this coral species to thermal stress does not come down to simple changes in *Symbiodinium* and/or the bacterial communities that associate with reef-building corals. This suggests that the physiological plasticity of the host and/or symbiotic components appears to play an important role in responding to ocean warming. The further study of host and symbiont physiology, both of *Symbiodinium* and prokaryotes, is of paramount importance in the context of global climate change, as mechanisms for rapid holobiont acclimatization will become increasingly important to the long-standing persistence of coral reefs.

Keywords: coral bleaching; acclimatization; symbiosis; thermal stress; global change

1. INTRODUCTION

Coral reefs are among the most biologically diverse and economically important ecosystems on the planet, providing ecological services that are vital to human society and industries through fisheries, coastal protection, pharmaceutical compounds and tourism [1]. The high productivity and structural complexity of coral reefs is derived and powered by the mutualistic association between corals and their symbiotic single-celled dinoflagellate algae (*Symbiodinium* Freudenthal [2]). Coral reefs worldwide, however, are among the most vulnerable ecosystems to global environmental change [3,4]. Both field and laboratory evidence reveal that corals are highly sensitive to thermal stress, with +1°C above long-term summer maxima driving mass coral bleaching (i.e. the loss of symbiotic dinoflagellates and/or their photosynthetic pigments) and consequently coral mortality [3,5,6]. If the thermal sensitivity of corals does not change, coral reefs face serious problems in scenarios where tropical seas may be as much as 2°C–4°C warmer by the end of this century [5]. The capacity of corals for acclimatization to heat stress is a critical component of their long-term survival. Understanding the thermal tolerance of corals and their dinoflagellate and prokaryotic symbionts, which altogether represent the holobiont [7], is therefore important to any predictions of how the future may unfold for coral reefs.

At fine scales, thermal history, both in the long- and short-term, has been shown to be a determinant in the response of corals to hyperthermal stress and bleaching [8–10]. By comparing the widespread thermal bleaching events that occurred on the Great Barrier Reef (GBR) in the Coral Sea in 1998 and 2002, Maynard *et al.* [8] detected a lower incidence of bleaching for three major coral genera (*Acropora*, *Pocillopora* and *Porites*) in 2002 when compared with 1998 on the same reefs, despite the higher solar irradiance observed during the 2002 thermal event. Since colony mortality was not high enough during the bleaching episode in 1998 for selection to explain the increased thermal tolerance observed during the 2002 thermal event, acclimatization was suggested as the potential cause of bleaching resistance [8]. Potentially, a long-term acclimatization response could have a basis in epigenetics, conferring a transcriptional response conducive to bleaching resistance. Though the role of epigenetics in coral stress response is currently unknown, there is evidence for the regulation of stress responses of plants via epigenetic mechanisms [11], and even transmission of epigenetic effects to subsequent generations [12]. Other evidence for the natural acclimatization of corals to thermal stress comes from studies conducted in the surroundings of the nuclear power plant near Nanwan Bay, Taiwan [13]. In 1988, the year the power plant began full operation, *Acropora grandis* samples taken near the hot water outlet of the nuclear power plant were completely bleached within 2 days of exposure to 33°C. Two years later, however, corals from the same area required 6 days of exposure to 33°C water for the onset of signs of bleaching. The protective effect of thermal preconditioning has also been shown experimentally, in the reef coral *Acropora aspera*, where corals exposed to brief heat stress insufficient to cause bleaching later

* Author for correspondence (rodriguez-lanetty@louisiana.edu).

Electronic supplementary material is available at http://dx.doi.org/10.1098/rspb.2011.1780 or via http://rspb.royalsocietypublishing.org.

Received 24 August 2011
Accepted 12 September 2011

resisted bleaching temperatures, maintaining symbiont densities, photopigments and quantum yield [9].

Attempts to understand the differences in the response of reef-building corals to warming oceans has focused almost entirely on genetic variation within the dinoflagellate symbiont, *Symbiodinium* [14–16]. There are two proposed mechanisms by which the composition of the *Symbiodinium* population hosted by a coral can potentially change: switching, in which existing symbionts are expulsed and novel symbionts are acquired from the environment, or shuffling (also referred as to shifting), in which existing types already *in hospite* change in relative abundance [17]. Shuffling from less tolerant to heat-resistant algal symbionts has been proposed as a means of adjusting to accelerating increases in sea water temperature [14–16,18]. However, the higher proportions of hosts harbouring heat-resistant algal symbionts after bleaching [15] could also be a result of differential survival of hosts containing the stress-resistant symbiont as opposed to changes of symbionts [19]. Sampayo *et al.* [20] monitored tagged colonies of *Stylophora pistillata* with sampling times spanning a bleaching event. Their results support differential mortality of corals hosting heat-sensitive symbionts as an explanation of an increase in the frequency of thermal-tolerant symbionts post-bleaching, not the beneficial shuffling of dinoflagellate symbionts [20]. Other work shows that bleached *Porites divaricata* challenged with heterologous *Symbiodinium* may transiently acquire symbionts from the water, but these novel symbioses are not maintained [21]. By contrast, the work of Jones *et al.* [22] supports acclimative shifts in *Symbiodinium*, finding that in tagged *Acropora millepora* colonies examined prior to and following a bleaching event, 71 per cent of surviving colonies which initially harboured a majority of heat-sensitive symbionts shifted to predominantly heat-tolerant symbionts after bleaching. Clearly, a general model for the role of symbiont shuffling and/or switching has yet to be established.

Another possibility is an advantageous change in the coral-associated bacterial community, resulting in the rapid generation of a more heat-resistant holobiont. This is based on the coral probiotic hypothesis [23], which states that corals form a symbiotic relationship with a diverse, metabolically active microbial population living on their surface and in their tissues [7,24–27], such that when environmental conditions are altered, the microbial biota undergo changes that aid the coral holobiont fitness [23]. However, unambiguous switching to entirely novel symbioses as a beneficial response to thermal stress has yet to be demonstrated. It is still unknown if the thermal tolerance observed in rapid acclimatization responses is also associated with a shift to heat-resistant symbionts in coral holobionts.

In this study, we asked whether short-term preconditioning of corals to thermal stress changed their dinoflagellate and bacterial communities to new configurations, thereby resulting in greater thermal tolerance for the host and symbionts.

2. MATERIAL AND METHODS

(a) *Sample collection and care*

Acropora millepora nubbins were collected from colonies on the reef flat in the vicinity of Heron Island (GBR), Queensland, Australia (23°33′ S, 151°54′ E) in June 2009.

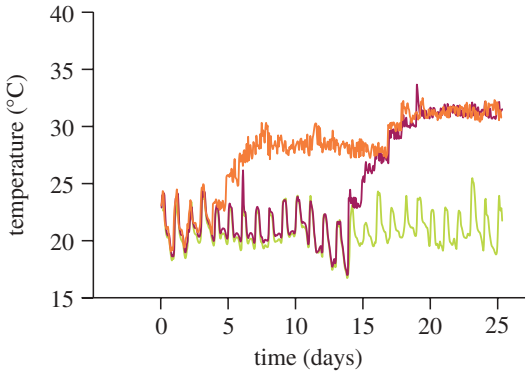

Figure 1. Temperature profiles of the thermal treatments with which *Acropora millepora* fragments were challenged. Preconditioned treatment comprised a 10 day pre-stress at 28°C prior to exposure to 31°C (orange line). Non-preconditioned treatment with no pre-stress period prior to exposure to 31°C (red line), and control (green line) where coral fragments were not challenged to increase of temperature. Temperature was brought to 31°C at 2°C per day.

Branches 6–8 cm long were cut and embedded in marine epoxy in cut-off 15 ml centrifuge tubes; a total of 72 coral fragments were allowed to recover for 20 days prior to the beginning of experimental manipulations.

(b) *Thermal stress experiments*

We tested the response of *A. millepora* to thermal preconditioning by exposing coral nubbins to 28°C (3°C below bleaching threshold) for 10 days, prior to challenging them with water temperatures of 31°C for 8 days (figure 1). In another treatment (non-preconditioned), corals were exposed to 31°C without prior exposure to the 28°C treatment. These two treatments were compared with control coral fragments that were exposed only to ambient-temperature reef flat water (21°C–22°C).

The experimental system comprised 15 l transparent tanks plumbed into flowing sea water, with four replicate tanks for each treatment (a total of 12 tanks). All tanks were operated as open systems and received water from the adjacent reef flat at a rate of 0.3–0.4 l min^{-1}, with additional flow provided by 250 l h^{-1} submersible pumps. Control treatments received ambient water with no temperature manipulation. The temperatures in the experimental tanks were increased at a rate of 2°C per day, with temperature changes taking place at 06.30. The water temperature in tanks was recorded throughout the experiment using HOBO Pro v. 2 Water Temperature Data Loggers (Onset, Pocasset, MA, USA). There were six nubbins per tank at the outset of the experiment (a total of 72 nubbins). One coral nubbin was collected for each experimental and control replicate tank at the following sampling times: day 0 (18 days prior to thermal stress), day 13 (at which point the preconditioned treatment had been exposed to 28°C preconditioning for 7 days), and days 19, 23 and 25 (in which treatment corals have been exposed to 31°C thermal challenge for 2, 6 and 8 days, respectively). Sampling was carried out at 17.00 on each indicated day.

(c) Symbiodinium *density and genetic identification*

For the determination of *Symbiodinium* cell densities per surface area, cell counts were performed using a Hirschman Neubauer improved haemocytometer (Hirschmann Laborgeräte, Eberstadt, Germany), with coral area assessed by a wax

coating method [28]. DNA extractions were performed using DNeasy Plant Mini Kit (QIAGEN, Valencia, CA, USA) from tissue collected 18 days prior to the start of thermal stress, as well as after 8 days of thermal challenge. *Symbiodinium* 28S rDNA was amplified and directly sequenced using primers 28S-forward (5′CCCGCTGAATTTAAGCATATAAGTAAG CGG-3′) and 28S-reverse (5′GTTAGACTCCTTGGTC CGTGTTTCAAGA-3′) [29]. PCR was performed in 25 µl reaction volumes, using 10 ng of DNA template, 10 µl GoTaq Green Master Mix (Promega, Madison, WI, USA), and 0.25 µM 28S-forward and 0.25 µM 28S-reverse primers. Thermocycling conditions consisted of a 5 min initial denaturation at 95°C, followed by 35 cycles of 94°C (30 s), 65°C (40 s) and 72°C (60 s), and a 10 min final extension at 72°C. PCR products were directly sequenced by the DNA Analysis Facility at Yale University (New Haven, CT, USA), using 28S-forward and 28S-reverse primers. Sequences were inspected and assembled using CodonCode Aligner v. 3.5.7 (CodonCode Corporation, Dedham, MA, USA). Sequences were identified by BLAST comparisons in NCBI GenBank.

The internal transcribed spacer region 2 (ITS2) rDNA was PCR-amplified using primers ITSintfor2 and ITS2CL AMP (5′GAATTGCAGAACTCCGTG-3′ and 5′CGCCC GCCGCGCCCCGCGCCCGTCCCGCCGCCCCCGCC CGGGATCCATATGCTTAAGTTCAGCGGGT-3′) [30], in 20 µl reactions consisting of 10 ng of DNA template, 10 µl GoTaq Green Master Mix (Promega) adjusted to 3.0 mM $MgCl_2$, 0.25 µM ITSintfor2, and 0.75 µM ITS2CLAMP. The touch-down PCR programme consisted of a 3 min initial denaturation at 92°C, 21 cycles of 92°C (30 s), 62°C (40 s) and 72°C (30 s), decreasing by 0.5°C each cycle, followed by 15 cycles with a 52°C annealing step, and a 10 min final extension at 72°C. Denaturing gradient gel electrophoresis (DGGE) was used to separate PCR products on a 45–80% gradient (8% acrylimide) [30]. Gels were run for 14 h at 100 V at a constant temperature of 60°C [30].

Excised bands were incubated for 24 h with shaking at room temperature in 30 µl nuclease-free water. The liquid portion of this mixture was recovered, ethanol-precipiated, washed with 70 per cent ethanol, and resuspended in 30 µl nuclease-free water. One microlitre of each band isolate solution was subsequently re-amplified for direct sequencing in a 20 µl reaction using 0.25 µM ITSintfor2 [30] and ITSRev (5′GGATCCATATGCTTAAGTTCAGCGGGT-3′) [31], 10 µl GoTaq Green Master Mix (Promega) adjusted to 3.0 mM $MgCl_2$, with a PCR programme consisting of a 3 min initial denaturation at 92°C, 35 cycles of 92°C (30 s), 52°C (40 s), 72°C (30 s) and a 10 min final extension at 72°C. Reamplification products were directly sequenced by the DNA Analysis Facility at Yale University (New Haven) using primer ITSintfor2 [30]. Sequences were examined using CodonCode Aligner v. 3.5.7 (CodonCode Corporation) and identified by BLAST comparisons in GenBank.

(d) *Bacterial community composition*

The bacterial community was profiled using RNA to assess the active microbial assemblage; in a short-course experiment, a DNA profile may have provided results not reflective of the actual active bacteria at a given time. RNA was extracted from snap frozen coral fragments sampled after 6 days at 31°C. RNA isolations were performed using Trizol reagent (Invitrogen), followed by RNeasy Mini Kit (QIAGEN). Total RNA (100 ng) was reverse-transcribed using a QuantiTect Reverse Transcription Kit (QIAGEN), with 1 µM of modified primer 907R (CCTACGGGDG GCWGCAG) [32]. Subsequently, PCR was performed on cDNA samples using modified primers 341F-*Clamp* [33] (CGCCCGCCGCGCCCCGCGCCCGTCCCGCCGCC CCCGCCCGCCTACGGGDGGCWGCAG) and 907R to amplify the 16S rRNA [32]. PCR was performed in 50 µl reaction volumes, using GoTaq Green Master Mix (Promega) adjusted to 2.5 mM $MgCl_2$, 0.25 µM 907R and 0.75 µM 341F-Clamp. The PCR programme consisted of a 5 min initial denaturation at 95°C, followed by 35 cycles of 95°C (30 s), 51°C (60 s) and 72°C (60 s), and a 7 min final extension. PCR products were run on DGGE using a 6% acrylimide denaturing gradient gel (30–65% gradient) for 14 h at 97 V at a constant temperature of 60°C.

Excised bands (processed as previously described) were subsequently re-amplified for direct sequencing in a 25 µl reaction using GoTaq Green Master Mix (Promega), 0.25 µM 907R and 0.25 µM 341F. The PCR programme consisted of a 5 min initial denaturation at 95°C, followed by 35 cycles of 95°C (30 s), 51°C (60 s) and 72°C (60 s), and a 7 min final extension. PCR products were directly sequenced by the DNA Analysis Facility at Yale University (New Haven) using the 907R primer. Sequences were examined using CodonCode Aligner v. 3.5.7 (CodonCode Corporation) and identified by BLAST comparisons in GenBank, and using the chimera-checked Greengenes database [34].

(e) *Statistical analysis and multivariate analysis*

Symbiodinium density data were analysed with one-way ANOVA with Tukey HSD, performed using the package Systat 13 (SYSTAT Inc, Evanston, IL, USA). DGGE gel images of the bacterial 16S rDNA were scored using Gel2k [35]. Gel band intensity was normalized and assigned to categories prior to multivariate analysis. Correspondence analysis (CA) of the categorical data was performed using the R package Vegan [36,37].

3. RESULTS

(a) *Thermal response:* Symbiodinium *density and composition*

Coral nubbins that were not exposed to the thermal preconditioning treatment suffered significant bleaching with exposure to 31°C water, as observed after 6 and 8 days of thermal challenge at 31°C ($p < 0.001$, one-way ANOVA with Tukey HSD; figure 2). *Symbiodinium* density decreased almost 80 per cent after 8 days of exposure to bleaching temperature. By contrast, preconditioned coral nubbins did not bleach with exposure to 31°C, maintaining dinoflagellate symbiont densities consistent with those observed in control coral fragments during the 8 days of thermal challenge ($p > 0.20$, one-way ANOVA with Tukey HSD; figure 2).

To determine whether the response to thermal stress was associated with changes in the composition of *Symbiodinium* strains, we directly sequenced the 28S rDNA and conducted DGGE analysis on the ITS2 of the resident *Symbiodinium* in the coral nubbins. The composition of *Symbiodinium* strains prior to thermal treatments and after 8 days of thermal challenge was revealed to be the same across treatments and controls, with all corals

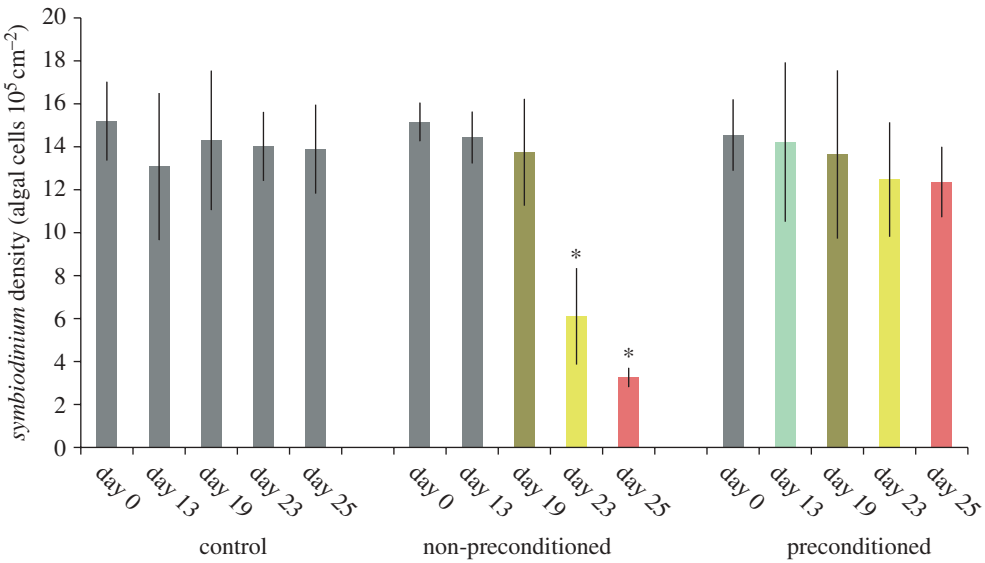

Figure 2. *Symbiodinium* density (algal cells per square centimetre) at five different times: 18 and 5 days prior to exposure at 31°C, and 2, 6 and 8 days during the exposure to 31°C. Asterisks indicate group is significantly different from controls ($p < 0.001$, one-way ANOVA with Tukey HSD, $n = 4$). Grey bars, ambient reef temperature; green bar, 7 days of 28°C preconditioning; dark green bars, 2 days at 31°C; yellow bars, 6 days at 31°C; pink bars, 8 days at 31°C.

maintaining an association with clade C3 *Symbiodinium* (electronic supplementary material, figure S1). With direct sequencing of 28S (GenBank accession number JF834208), no background sequences were detected.

(b) *Thermal response by the bacterial community*

The DGGE analysis of PCR-amplified bacterial 16S rRNA fragments showed no differences in the composition of the bacterial community associated with preconditioned, non-preconditioned and control coral nubbins, with the exception of one control coral containing an additional band (electronic supplementary material, figure S2). Dominant banding patterns and bacterial types that were ubiquitous across treatments were sequenced, revealing that the majority of abundant sequences were Gammaproteobacteria of high identity (95–96%) to *Spongiobacter* spp. sequences. However, differences occurred in the relative intensity of 16S rRNA-DGGE bands, which were used as a rough proxy of the relative abundance. Analysis of gel banding-intensity patterns using CA revealed an effect of temperature on both the non-preconditioned and preconditioned corals after 6 days at 31°C, relative to controls (figure 3). Nevertheless, this temperature effect was similar on both non-preconditioned (bleached) and preconditioned (thermal acclimatized) corals.

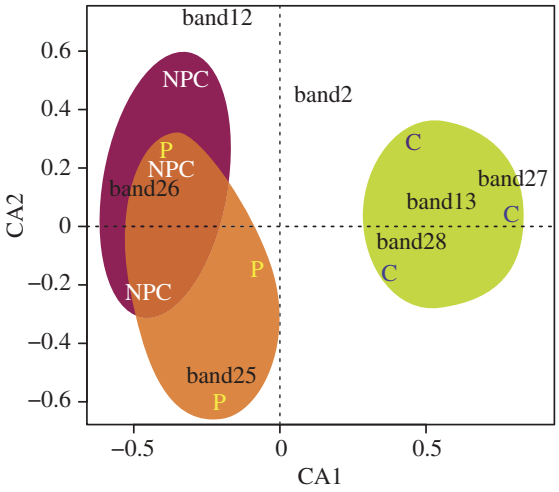

Figure 3. Correspondence analysis (CA) of reverse-transcribed bacterial 16S rRNA-DGGE banding patterns (treatment samples, as indicated in the legend, $n = 3$). DGGE bands incorporated in the CA through relative abundance are shown. The bacterial community was profiled from RNA to assess the active microbial assemblage. CA1 explains 58.5% of variation; CA2 explains 19.3%. NPC, non-preconditioned (red colour); P, preconditioned (orange colour); C, control (green colour).

4. DISCUSSION

The findings of this study revealed that the acquired tolerance of preconditioned *Acropora millepora* corals to thermal stress was not owing to changes in the make-up of their symbionts. There were no changes detected in *Symbiodinium* strains associated with the thermal tolerance response described in this experiment. Even more, no change in the dominant members of the bacterial community was detected, and the community structures, based on relative abundance of bacteria, were largely similar across bleached non-preconditioned corals and non-bleaching preconditioned corals. Therefore, our results indicate that the rapid acclimatization of *A. millepora* corals to thermal stress did not come down to simple changes in *Symbiodinium* and/or the bacterial communities that associate with reef-building corals.

Changes in symbiont type via shuffling would seem unlikely, as the corals hosted a single *Symbiodinium* type, but the point of emphasis remains that even with one detectable symbiont type, preconditioned corals still exhibit evidence of acclimatization. It bears mentioning that the DGGE implemented is capable of detecting broad changes in the symbiont community, but it is unable to detect *Symbiodinium* present at less than

5–10% of the community [38,39]. In terms of switching, host-symbiont specificity would also seriously hinder the acquisition of novel symbiont types, both in terms of symbiont uptake and proliferation [40–42]. But even with a lack of specificity, the complete replacement of dominant symbionts by another type (switching) would take in excess of one month, according to a *Symbiodinium* population model for corals proposed by Jones & Yellowlees [43]. This stands in contrast to the thermal acclimation which occurred in only 10 days in this experiment, as well as in the work of Middlebrook *et al.* [9], with 48 h preconditioning regimes one and two weeks prior to thermal challenge. These timescales are incompatible with a symbiont type switch. In accordance with this, our molecular genotyping of *Symbiodinium* revealed the same single symbiont type both at the outset and at the end of the experiment.

Likewise, it has been recently proposed that changes of the bacterial community in response to environmental stressors could also provide tolerance to changing environmental conditions much more rapidly than host evolution—this is referred to as to 'the Coral Probiotic hypothesis' [23]. In *A. millepora*, shifts in resident bacteria have been shown during bleaching, with a change in the community shifting from a healthy community of bacteria dominated by *Spongiobacter* spp. to one dominated by *Vibrio* spp. during bleaching [24,44]. However, in the present experiment, a dramatic change in members of the bacterial community was not found in either preconditioned or non-preconditioned corals. While we indeed detected some changes in the apparent relative abundance of bacterial strains in response to an increase of temperature compared with controls, the changes were similar between preconditioned (acclimatized) and non-preconditioned (bleached) corals, indicating no correlation between a change of the bacterial community and the thermal tolerance response.

One possibility that could explain the lack of a bacterial partner shift in this experiment is that the corals already possessed a community of bacteria able to cope with the fluctuating temperatures as a result of prior stress on the reef, as back reef environments usually experience greater temperature extremes and fluctuations than forereef environments [45]. Intriguingly, the effects of thermal acclimation on the bacterium *Escherichia coli* defy a single model, with acclimation competitively beneficial in some cases, but not in others, as shown experimentally [46]. Another consideration is that a mass bleaching event is one of an entire community, presumably with bacterial fauna present from the entire heat-stressed vicinity. However, in this experiment, the flowing sea water originated from a reef flat that was not experiencing hyperthermal stress, eliminating a potential source of bacteria that may colonize corals during natural bleaching events. This could explain why this experiment did not see a bacterial community flux as observed in other studies [24,44]. Similar to our results, Salerno *et al.* [47] found no systematic changes in the microbial community composition of *Porites compressa* as a result of a 6 day treatment of 1°C above ambient summer temperature.

Although symbionts are clearly of fundamental importance, the idea that the thermal tolerance of corals resides almost entirely within changes of symbiont types with different physiologies has been questioned [10,48,49]. Our findings instead suggest that the physiological plasticity of one or more members of the coral holobiont plays, in a timely and beneficial way, an important role in the acclimatization response to a rapid change of temperature. This is, to our knowledge, the first study that shows clearly the capacity of corals to tolerate thermal stress through a mechanism based on genotype-independent phenotypic change.

In addition to the potential of *Symbiodinium* population shifts, the consideration of physiological acclimatization is important both in the host and the symbionts. Physiological acclimatization to heat stress has been previously documented in corals, and, alongside the role of the dinoflagellate symbiont, is held to be a significant part of the response to heat stress [50]. In *Montastraea franksi*, exposure to elevated temperatures results in the upregulation of HSP70 after 6 h, with a return to control levels after continued exposure for 12 h, and another increase in expression after 48 h of heat stress [51]. Additionally, Gates & Edmunds [51] suggest a relationship between corals with high protein turnover and an increased capacity for thermal acclimatization. This relationship is based on evidence from *Mytilus edulis*, in which mussels with higher rates of protein turnover have been shown to acclimatize faster than those with lower rates of protein turnover [52,53]. As shown with transcriptome analysis via cDNA microarrays, the aposymbiotic larvae of *A. millepora* exhibit a marked response in gene expression when heat-stressed, including the rapid upregulation of three heat-shock proteins and a fluorescent protein [54]. Host physiology and the ability to induce stress response proteins has been suggested to play a role in resistance to heat stress in the case of *Porites cylindrica*, in addition to a heat-resistant symbiont [55]. *Pocillopora damicornis* of a host genotype originating from a non-upwelling area showed greater thermal tolerance experimentally than another genotype, originating from an upwelling region of lower thermal stress, suggesting the possible importance of the host in thermal tolerance or the effects of long-term acclimatization to thermal stress [56].

Additionally, the prior experience of the host has been shown to be of great influence, even with differential exposure of stress within a colony. Brown *et al.* [57] found that the west faces of *Goniastrea aspera* colonies resisted bleaching during natural heat stress, whereas the east faces bleached. The west faces had been preconditioned via prior solar irradiance, conferring thermal tolerance without a change in dinoflagellate symbiont type [57]. Although the source of acclimatization was reported to be that of solar irradiance, a resultant increase in thermal tolerance is mechanistically possible as stress responses are often unspecific [58]. Environmental stressors often coincide, and a general response has the advantage that a single stimulus mounts a response to potentially multiple simultaneous environmental conditions [58].

The contribution of the host to thermal tolerance is once again highlighted in a reciprocal transplant experiment with *Porites lobata* between genetically distinct populations of corals from back reef and forereef environments [49]. The host origin and associated genotype were the major determinants of ubiquitin-conjugated protein concentration, whereas *Symbiodinium* populating the corals were genetically indistinguishable [49]. Higher levels of ubiquitin-conjugated proteins were consistently

found in colonies originating from the highly fluctuating back reef environment, both prior to and following transplantation, indicating a distinct physiological difference associated with colony genotype [49]. The differentiation in host populations between forereef and back reef sites calls into focus the potential for selection for physiological acclimatization to stress, given the disparate thermal regimens experienced by the two populations [49]. Thompson & Van Woesik [59] add additional credence to an argument for host selection in response to thermal stress, but in the larger context of differential mortality and selection in response to thermal stress, as sites with historically high variability in temperature and solar irradiance resisted heat stress. This stands at odds with the conclusions drawn by Maynard *et al.* [8], finding that differential mortality did not explain thermal tolerance, but their sampling times may have not captured all post-bleaching mortality [59].

Although a response of the cnidarian host to stress is one mechanism of acclimatization, there remain other possibilities, including that of physiological acclimatization of the dinoflagellate symbiont. In culture, *Symbiodinium* cells have been shown to decrease their cellular chlorophyll *a* in response to supersaturating irradiance [60], a photoacclimation response common to many microalgae [61]. Warner *et al.* [45] found differences in photoacclimation between the symbionts of forereef and back reef coral species, with the *Symbiodinium* cells in the forereef *Montastraea annularis* less thermally tolerant than those in the back reef *Siderastrea radians*. While symbionts from both coral species induce non-photochemical quenching (NPQ) rapidly in response to elevated temperatures, the increase of NPQ is higher in the *Symbiodinium* of *S. radians*. Other work has identified NPQ as a mechanism by which *Symbiodinium* can dissipate excess light energy in response to thermal stress that causes a loss in the functionality of photosystem II reaction centres [62]. Still, there remain many questions about the mechanisms and roles of acclimatization in *Symbiodinium*.

Coral bleaching is a symptom of host–symbiont disequilibrium, and as such is potentially a consequence of multiple aetiologies, with perhaps multiple modes of acclimatization under different circumstances and timeframes. Understanding how corals can adjust their thermal sensitivity in the context of global climate change continues to be important in understanding the long-term persistence of coral reefs under global change.

We are grateful to Dr Paulina Kaniewska, Alison Darling, Rachel Middlebrook, Camila Granados-Cifuentes, Robert Quinn, and the staff of the Heron Island Research Station for their assistance in the field and laboratory work on this project. We also thank Camila Granados-Cifuentes, Tanya Brown and two anonymous reviewers for their comments on early versions of this manuscript. This research was funded by an NSF-OCE grant (0851123) awarded to M.R.-L.

REFERENCES

1 Moberg, F. & Folke, C. 1999 Ecological goods and services of coral reef ecosystems. *Ecol. Econ.* **29**, 215–233. (doi:10.1016/S0921-8009(99)00009-9)
2 Freudenthal, H. D. 1962 *Symbiodinium* gen. nov. and *Symbiodinium microadriaticum* sp. nov., a zooxanthella: taxonomy, life cycle, and morphology. *J. Eukaryot. Microbiol.* **9**, 45–52. (doi:10.1111/j.1550-7408.1962.tb02579.x)
3 Hoegh-Guldberg, O. 1999 Climate change, coral bleaching and the future of the world's coral reefs. *Mar. Freshwater Res.* **50**, 839–866. (doi:10.1071/MF99078)
4 Hughes, T. P. *et al.* 2003 Climate change, human impacts, and the resilience of coral reefs. *Science* **301**, 929–933. (doi:10.1126/science.1085046)
5 Donner, S. D., Skirving, W. J., Little, C. M., Oppenheimer, M. & Hoegh-Guldberg, O. 2005 Global assessment of coral bleaching and required rates of adaptation under climate change. *Glob. Change Biol.* **11**, 2251–2265. (doi:10.1111/j.1365-2486.2005.01073.x)
6 Hoegh-Guldberg, O. *et al.* 2007 Coral reefs under rapid climate change and ocean acidification. *Science* **318**, 1737–1742. (doi:10.1126/science.1152509)
7 Rohwer, F., Seguritan, V., Azam, F. & Knowlton, N. 2002 Diversity and distribution of coral-associated bacteria. *Mar. Ecol. Prog. Ser.* **243**, 1–10. (doi:10.3354/meps243001)
8 Maynard, J. A., Anthony, K. R. N., Marshall, P. A. & Masiri, I. 2008 Major bleaching events can lead to increased thermal tolerance in corals. *Mar. Biol.* **155**, 173–182. (doi:10.1007/s00227-008-1015-y)
9 Middlebrook, R., Hoegh-Guldberg, O. & Leggat, W. 2008 The effect of thermal history on the susceptibility of reef-building corals to thermal stress. *J. Exp. Biol.* **211**, 1050–1056. (doi:10.1242/jeb.013284)
10 Oliver, T. A. & Palumbi, S. R. 2011 Do fluctuating temperature environments elevate coral thermal tolerance? *Coral Reefs* **30**, 429–440. (doi:10.1007/s00338-011-0721-y)
11 Chinnusamy, V. & Zhu, J. K. 2009 Epigenetic regulation of stress responses in plants. *Curr. Opin. Plant Biol.* **12**, 133–139. (doi:10.1016/j.pbi.2008.12.006)
12 Molinier, J., Ries, G., Zipfel, G. & Hohn, B. 2006 Transgeneration memory of stress in plants. *Nature* **442**, 1046–1049. (doi:10.1038/nature05022)
13 Fang, L. S., Huang, S. P. & Lin, K. L. 1997 High temperature induces the synthesis of heat-shock proteins and the elevation of intracellular calcium in the coral *Acropora grandis*. *Coral Reefs* **16**, 127–131. (doi:10.1007/s003380050066)
14 Baker, A. C. 2001 Reef corals bleach to survive change. *Nature* **411**, 765–766. (doi:10.1038/35081151)
15 Baker, A. C., Starger, C. J., McClanahan, T. R. & Glynn, P. W. 2004 Corals' adaptive response to climate change. *Nature* **430**, 741. (doi:10.1038/430741a)
16 Berkelmans, R. & Van Oppen, M. J. H. 2006 The role of zooxanthellae in the thermal tolerance of corals: a 'nugget of hope' for coral reefs in an era of climate change. *Proc. R. Soc. B* **273**, 2305–2312. (doi:10.1098/rspb.2006.3567)
17 Stat, M., Carter, D. & Hoegh-Guldberg, O. 2006 The evolutionary history of *Symbiodinium* and scleractinian hosts: symbiosis, diversity, and the effect of climate change. *Perspect. Plant Ecol. Evol. Syst.* **8**, 23–43. (doi:10.1016/j.ppees.2006.04.001)
18 Buddemeier, R. W. & Fautin, D. G. 1993 Coral bleaching as an adaptive mechanism: a testable hypothesis. *Bioscience* **43**, 320–326. (doi:10.2307/1312064)
19 Weis, V. M. 2010 The susceptibility and resilience of corals to thermal stress: adaptation, acclimatization or both? *Mol. Ecol.* **19**, 1515–1517. (doi:10.1111/j.1365-294X.2010.04575.x)
20 Sampayo, E., Ridgway, T., Bongaerts, P. & Hoegh-Guldberg, O. 2008 Bleaching susceptibility and mortality of corals are determined by fine-scale differences in symbiont type. *Proc. Natl Acad. Sci. USA* **105**, 10 444–10 449. (doi:10.1073/pnas.0708049105)
21 Coffroth, M. A., Poland, D. M., Petrou, E. L., Brazeau, D. A. & Holmberg, J. C. 2010 Environmental symbiont

acquisition may not be the solution to warming seas for reef-building corals. *PLoS ONE* **5**, e13258. (doi:10.1371/journal.pone.0013258)
22 Jones, A. M., Berkelmans, R., Van Oppen, M. J. H., Mieog, J. C. & Sinclair, W. 2008 A community change in the algal endosymbionts of a scleractinian coral following a natural bleaching event: field evidence of acclimatization. *Proc. R. Soc. B* **275**, 1359–1365. (doi:10.1098/rspb.2008.0069)
23 Reshef, L., Koren, O., Loya, Y., Zilber-Rosenberg, I. & Rosenberg, E. 2006 The coral probiotic hypothesis. *Environ. Microbiol.* **8**, 2068–2073. (doi:10.1111/j.1462-2920.2006.01148.x)
24 Bourne, D., Iida, Y., Uthicke, S. & Smith-Keune, C. 2008 Changes in coral-associated microbial communities during a bleaching event. *ISME J.* **2**, 350–363. (doi:10.1038/ismej.2007.112)
25 Ritchie, K. B. 2006 Regulation of microbial populations by coral surface mucus and mucus-associated bacteria. *Mar. Ecol. Prog. Ser.* **322**, 1–14. (doi:10.3354/meps322001)
26 Rohwer, F., Breitbart, M., Jara, J., Azam, F. & Knowlton, N. 2001 Diversity of bacteria associated with the Caribbean coral *Montastraea franksi*. *Coral Reefs* **20**, 85–91. (doi:10.1007/s003380100138)
27 Sweet, M. J., Croquer, A. & Bythell, J. C. 2011 Bacterial assemblages differ between compartments within the coral holobiont. *Coral Reefs* **30**, 39–52. (doi:10.1007/s00338-010-0695-1)
28 Stimson, J. & Kinzie, R. A. 1991 The temporal pattern and rate of release of zooxanthellae from the reef coral *Pocillopora damicornis* (Linnaeus) under nitrogen-enrichment and control conditions. *J. Exp. Mar. Biol. Ecol.* **153**, 63–74. (doi:10.1016/S0022-0981(05)80006-1)
29 Zardoya, R., Costas, E., Lopezrodas, V., Garridopertierra, A. & Bautista, J. M. 1995 Revised dinoflagellate phylogeny inferred from molecular analysis of large-subunit ribosomal RNA gene sequences. *J. Mol. Evol.* **41**, 637–645. (doi:10.1007/BF00175822)
30 LaJeunesse, T. C. & Trench, R. K. 2000 Biogeography of two species of *Symbiodinium* (Freudenthal) inhabiting the intertidal sea anemone *Anthopleura elegantissima* (Brandt). *Biol. Bull. (Woods Hole)* **199**, 126–134. (doi:10.2307/1542872)
31 Coleman, A. W., Suarez, A. & Goff, L. J. 1994 Molecular delineation of species and syngens in Volvocacean green algae (Chlorophyta). *J. Phycol.* **30**, 80–90. (doi:10.1111/j.0022-3646.1994.00080.x)
32 Muyzer, G., Brinkhoff, T., Nübel, U., Santegoeds, C., Schäfer, H. & Wawer, C. 1998 Denaturing gradient gel electrophoresis (DGGE) in microbial ecology. In *Molecular microbial ecology manual* (eds A. D. L. Akkermans, J. D. Van Elsas & F. J. de Bruijn), pp. 1–27. Dordrecht, The Netherlands: Kluwer Academic Publishers.
33 Muyzer, G., Dewaal, E. C. & Uitterlinden, A. G. 1993 Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl. Environ. Microbiol.* **59**, 695–700.
34 DeSantis, T. Z. *et al.* 2006 Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl. Environ. Microbiol.* **72**, 5069–5072. (doi:10.1128/aem.03006-05)
35 Norland, S. 2004 *GEL2k*, v. 1.2.6. Bergen, Norway: University of Bergen. See http://folk.uib.no/ninsn/gel2k
36 Oksanen, J. F., Blanchet, F. G., Kindt, R., Legendre, P., O'Hara, R. B., Simpson, G. L., Solymos, M. P., Stevens, H. H. & Wagner, H. 2011 VEGAN: community ecology package. R package version 1.17-8. See http://CRAN.R-project.org/package=vegan/.
37 R Development Core Team 2011 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
38 Fabricius, K. E., Mieog, J. C., Colin, P. L., Idip, D. & Van Oppen, M. J. H. 2004 Identity and diversity of coral endosymbionts (zooxanthellae) from three Palauan reefs with contrasting bleaching, temperature and shading histories. *Mol. Ecol.* **13**, 2445–2458. (doi:10.1111/j.1365-294X.2004.02230.x)
39 Thornhill, D. J., LaJeunesse, T. C., Kemp, D. W., Fitt, W. K. & Schmidt, G. W. 2006 Multi-year, seasonal genotypic surveys of coral-algal symbioses reveal prevalent stability or post-bleaching reversion. *Mar. Biol.* **148**, 711–722. (doi:10.1007/s00227-005-0114-2)
40 Rodriguez-Lanetty, M., Krupp, D. A. & Weis, V. M. 2004 Distinct ITS types of symbiodinium in Clade C correlate with cnidarian/dinoflagellate specificity during onset of symbiosis. *Mar. Ecol. Prog. Ser.* **275**, 97–102. (doi:10.3354/meps275097)
41 Rodriguez-Lanetty, M., Wood-Charlson, E. M., Hollingsworth, L. L., Krupp, D. A. & Weis, V. M. 2006 Temporal and spatial infection dynamics indicate recognition events in the early hours of a dinoflagellate/coral symbiosis. *Mar. Biol.* **149**, 713–719. (doi:10.1007/s00227-006-0272-x)
42 Schoenberg, D. & Trench, R. 1980 Genetic variation in *Symbiodinium* (=*Gymnodinium*) *microadriaticum* Freudenthal, and specificity in its symbiosis with marine invertebrates. III. Specificity and infectivity of *Symbiodinium microadriaticum*. *Proc. R. Soc. Lond. B* **207**, 445–460. (doi:10.1098/rspb.1980.0033)
43 Jones, R. J. & Yellowlees, D. 1997 Regulation and control of intracellular algae (=zooxanthellae) in hard corals. *Phil. Trans. R. Soc. Lond. B* **352**, 457–468. (doi:10.1098/rstb.1997.0033)
44 Bourne, D. G., Garren, M., Work, T. M., Rosenberg, E., Smith, G. W. & Harvell, C. D. 2009 Microbial disease and the coral holobiont. *Trends Microbiol.* **17**, 554–562. (doi:10.1016/j.tim.2009.09.004)
45 Warner, M. E., Fitt, W. K. & Schmidt, G. W. 1996 The effects of elevated temperature on the photosynthetic efficiency of zooxanthellae *in hospite* from four different species of reef coral: a novel approach. *Plant Cell Environ.* **19**, 291–299. (doi:10.1111/j.1365-3040.1996.tb00251.x)
46 Bennett, A. F. & Lenski, R. E. 1997 Evolutionary adaptation to temperature. VI. Phenotypic acclimation and its evolution in *Escherichia coli*. *Evolution* **51**, 36–44. (doi:10.2307/2410958)
47 Salerno, J. L., Reineman, D. R., Gates, R. D. & Rappé, M. S. 2010 The effect of a sublethal temperature elevation on the structure of bacterial communities associated with the coral *Porites compressa*. *J. Mar. Biol.* **2011**, 9. (doi:10.1155/2011/969173)
48 Baird, A. & Maynard, J. A. 2008 Coral adaptation in the face of climate change. *Science* **320**, 315–316. (doi:10.1126/science.320.5874.315)
49 Barshis, D. J., Stillman, J. H., Gates, R. D., Toonen, R. J., Smith, L. W. & Birkeland, C. 2010 Protein expression and genetic structure of the coral *Porites lobata* in an environmentally extreme Samoan back reef: does host genotype limit phenotypic plasticity? *Mol. Ecol.* **19**, 1705–1720. (doi:10.1111/j.1365-294X.2010.04574.x)
50 Baird, A. H., Bhagooli, R., Ralph, P. J. & Takahashi, S. 2009 Coral bleaching: the role of the host. *Trends Ecol. Evol.* **24**, 16–20. (doi:10.1016/j.tree.2008.09.005)
51 Gates, R. D. & Edmunds, P. J. 1999 The physiological mechanisms of acclimatization in tropical reef corals. *Am. Zool.* **39**, 30–43.
52 Hawkins, A. 1991 Protein turnover: a functional appraisal. *Funct. Ecol.* **5**, 222–233. (doi:10.2307/2389260)

53 Hawkins, A., Wilson, I. & Bayne, B. 1987 Thermal responses reflect protein turnover in *Mytilus edulis* L. *Funct. Ecol.* **1**, 339–351. (doi:10.2307/2389790)
54 Rodriguez-Lanetty, M., Harii, S. & Hoegh-Guldberg, O. 2009 Early molecular responses of coral larvae to hyperthermal stress. *Mol. Ecol.* **18**, 5101–5114. (doi:10.1111/j.1365-294X.2009.04419.x)
55 Fitt, W. K. *et al.* 2009 Response of two species of Indo-Pacific corals, *Porites cylindrica* and *Stylophora pistillata*, to short-term thermal stress: the host does matter in determining the tolerance of corals to bleaching. *J. Exp. Mar. Biol. Ecol.* **373**, 102–110. (doi:10.1016/j.jembe.2009.03.011)
56 D'Croz, L. & Mate, J. L. 2004 Experimental responses to elevated water temperature in genotypes of the reef coral *Pocillopora damicornis* from upwelling and non-upwelling environments in Panama. *Coral Reefs* **23**, 473–483. (doi:10.1007/s00338-004-0397-7)
57 Brown, B. E., Dunne, R. P., Warner, M. E., Ambarsari, I., Fitt, W. K., Gibb, S. W. & Cummings, D. G. 2000 Damage and recovery of photosystem II during a manipulative field experiment on solar bleaching in the coral *Goniastrea aspera*. *Mar. Ecol. Prog. Ser.* **195**, 117–124. (doi:10.3354/meps195117)
58 López-Maury, L., Marguerat, S. & Bähler, J. 2008 Tuning gene expression to changing environments: from rapid responses to evolutionary adaptation. *Nat. Rev. Genet.* **9**, 583–593. (doi:10.1038/nrg2398)
59 Thompson, D. M. & Van Woesik, R. 2009 Corals escape bleaching in regions that recently and historically experienced frequent thermal stress. *Proc. R. Soc. B* **276**, 2893–2901. (doi:10.1098/rspb.2009.0591)
60 Robison, J. D. & Warner, M. E. 2006 Differential impacts of photoacclimation and thermal stress on the photobiology of four different phylotypes of *Symbiodinium* (Pyrrhophyta). *J. Phycol.* **42**, 568–579. (doi:10.1111/j.1529-8817.2006.00232.x)
61 Falkowski, P. G. & Raven, J. A. 1997 *Aquatic photosynthesis*. Oxford, UK: Blackwell.
62 Hill, R., Larkum, A. W. D., Frankart, C., Kuhl, M. & Ralph, P. J. 2004 Loss of functional photosystem II reaction centres in zooxanthellae of corals exposed to bleaching conditions: using fluorescence rise kinetics. *Photosynth. Res.* **82**, 59–72. (doi:10.1023/B:PRES.0000040444.41179.09)