Bleaching susceptibility and mortality of corals are determined by fine-scale differences in symbiont type

E. M. Sampayo*, T. Ridgway, P. Bongaerts, and O. Hoegh-Guldberg

Centre for Marine Studies, The University of Queensland, St. Lucia QLD 4072, Australia

Edited by William C. Clark, Harvard University, Cambridge, MA, and approved May 8, 2008 (received for review August 25, 2007)

Coral bleaching has been identified as one of the major contributors to coral reef decline, and the occurrence of different symbionts determined by broad genetic groupings (clades A–H) is commonly used to explain thermal responses of reef-building corals. By using *Stylophora pistillata* as a model, we monitored individual tagged colonies *in situ* over a two-year period and show that fine level genetic variability within clade C is correlated to differences in bleaching susceptibility. Based on denaturing gradient gel electrophoresis of the internal transcribed spacer region 2, visual bleaching assessments, symbiont densities, host protein, and pulse amplitude modulated fluorometry, we show that subcladal types C78 and C8/a are more thermally tolerant than C79 and C35/a, which suffered significant bleaching and postbleaching mortality. Although additional symbiont types were detected during bleaching in colonies harboring types C79 and C35/a, all colonies reverted back to their original symbionts postbleaching. Most importantly, the data propose that the differential mortality of hosts harboring thermally sensitive versus resistant symbionts rather than symbiont shuffling/switching within a single host is responsible for the observed symbiont composition changes of coral communities after bleaching. This study therefore highlights that the use of broad cladal designations may not be suitable to describe differences in bleaching susceptibility, and that differential mortality results in a loss of both symbiont and host genetic diversity and therefore represents an important mechanism in explaining how coral reef communities may respond to changing conditions.

climate change | coral reefs | *Symbiodinium* | zooxanthellae | *Stylophora*

During the past 500,000 years coral communities have shown incredible persistence in taxonomic composition and diversity, yet there has been an unprecedented decline and change in coral community composition in the past 30 years (1, 2). These declines are driven by a number of human-induced disturbances (3, 4), but climate change is now regarded as one of the most serious long-term threats to the sustainability of coral reefs (5, 6). Climate-driven changes have impacted our oceans through increased sea surface temperatures (SSTs) but now extend further to include changes in ocean chemistry (6–8). Increased SSTs have been directly linked to the occurrence of mass coral bleaching during which the symbiotic relationship between corals and dinoflagellates of the genus *Symbiodinium* breaks down (2, 5, 7). Highly variable patterns of bleaching have been recorded between and within geographic locations, host genera (7, 9, 10), and even within single species (11, 12). This variable response has been related to host factors (e.g., 13–15) and/or the presence of genetically distinct *Symbiodinium* types residing within the coral host.

Bleaching patterns in *Montastraea annularis*, for example, are linked to cladal zonation patterns (16, 17). Also, *Pocillopora damicornis* colonies harboring clade C are more susceptible to bleaching than those containing clade D (12, 18). Recent reports propose that clade D is generally more thermally tolerant than clade C (18–21), which is of particular concern given that most coral species in the Indo-Pacific region associate with clade C (22–24). There is, however, increasing evidence that symbiont identification to the cladal level is too broad and does not overlap with physiological differences (e.g., 25). Finer level classification to subcladal types has unraveled biologically relevant patterns such as host specificity, niche diversification, and physiological differences between subcladal types (22, 25–29). As such, the continued use of cladal designations may have delayed our understanding of how symbiont diversity plays a role in determining the bleaching response of reef-building corals.

The predictions of an increased frequency and severity of bleaching events in the coming decades (5, 7) will significantly impact tropical near-shore communities. It is therefore imperative that we fully understand the mechanisms driving coral community change. The Adaptive Bleaching Hypothesis (ABH; 30) has been proposed as a mechanism whereby reef-building corals switch or shuffle their algal symbionts for more tolerant varieties during bleaching and has been supported by studies that show increased thermal tolerance of novel associations (21) or that note increases of clade D within the community postbleaching (19, 31). However, the mode by which community changes occur in response to environmental perturbations may not only be through the displacement of resident symbionts on the colony level. Changes can also occur through differential mortality of colonies hosting sensitive versus tolerant symbionts but, because of the scarcity of studies that monitor individual colonies through natural bleaching episodes, data that can address this fundamental question are currently limited.

The widespread Indo-Pacific coral *Stylophora pistillata* is an interesting case in point in that it harbors multiple distinct subcladal C types on the southern Great Barrier Reef (sGBR) (29). Given that the bleaching response can be a function of host and symbiont factors, the fact that multiple symbiont types occur within the same coral species makes *S. pistillata* an ideal test candidate because it reduces possible confounding host effects. Thus, the genetic and physiological responses of *in situ* tagged individuals of *S. pistillata* were monitored over a two-year period, which included a natural bleaching event. This study therefore not only directly compares the physiological response of different subcladal types (of a single clade) within the host during a natural bleaching event, but also addresses the fundamental question of how symbiont and host communities may change in response to environmental disturbance.

Results

In total 46 adult colonies of *S. pistillata* were tagged at Heron Island on the southern Great Barrier Reef (sGBR) in March 2005 at shallow (3–6 m) and deep (15–18 m) reef locations. Individuals were repeatedly sampled for physiological measurements and genetic analysis (see *Materials and Methods*) at each

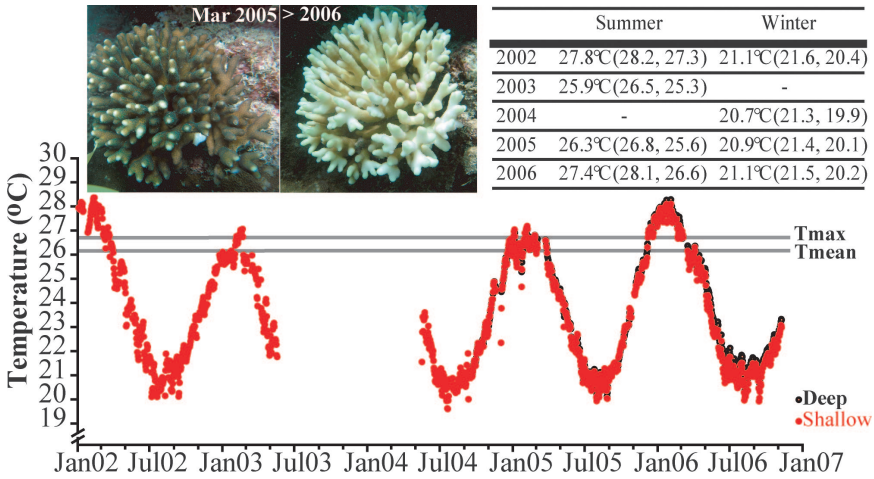
Author contributions: E.M.S. and T.R. designed research; E.M.S., T.R., and P.B. performed research; E.M.S. contributed new reagents/analytic tools; E.M.S. analyzed data; and E.M.S., T.R., and O.H.-G. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. EU807997–EU808004).

*To whom correspondence should be addressed. E-mail: e.sampayo@cms.uq.edu.au.



	Summer	Winter
2002	27.8°C(28.2, 27.3)	21.1°C(21.6, 20.4)
2003	25.9°C(26.5, 25.3)	-
2004	-	20.7°C(21.3, 19.9)
2005	26.3°C(26.8, 25.6)	20.9°C(21.4, 20.1)
2006	27.4°C(28.1, 26.6)	21.1°C(21.5, 20.2)

Fig. 1. Seawater temperatures recorded from 2002 to 2006 at Heron Island on the southern Great Barrier Reef (June 2003 to June 2004, no data available). (*Inset Right*) Average (maximum, minimum) summer and winter temperatures. Water temperatures did not differ significantly between the deep (15–18 m) and shallow (3–6 m), but regional bleaching was evident in 2002 and 2006 when the long-term summer average (Tmean) or maximum (Tmax) was exceeded for prolonged periods. (*Inset Left*) Tagged *Stylophora pistillata* colony in March 2005 (healthy) and March 2006 (bleached).

of four time points: March 2005, November 2005, March 2006, and November 2006.

Physical Data. Summer 2006 water temperatures reached temperatures similar to those of the summer 2002 GBR bleaching event (Fig. 1), with the average temperature difference between a bleaching and a "normal" summer being ≈1.5°C. Temperatures exceeded the long-term summer maxima in early December 2005, resulting in widespread reports of coral bleaching on the sGBR by late December 2005. No significant difference was observed in mean water temperature between the deep and shallow reef locations (Fig. 1; $P = 0.138$, t value $= 1.485$, $n = 697$), despite the daily temperature fluctuations (difference between maximum and minimum) in the shallow being higher (≈1°C) than in the deep reef locations (≈0.4°C).

The effect of irradiance was also tested because it may exacerbate the bleaching response (7, 15, 32). Colonies tagged in deep or shallow-cryptic locations received low average daily irradiance of ≈50 μmol quanta·s^{-1} and levels of bleaching were similar between these groups (deep: 54%, $n = 13$; shallow-cryptic: 40%, $n = 15$). Shallow-exposed colonies experienced average daily irradiance levels of ≈350–650 μmol quanta·s^{-1}, but only recorded a slightly higher bleaching occurrence (55.6%, $n = 18$). No significant effect of light was found between categories ($G = 5.81$, 2; $P = 0.55$) suggesting that the observed bleaching response was predominantly driven by increased water temperature under reduced influence of light.

Genetic Data. Denaturing gradient gel electrophoresis of the internal transcribed spacer region 2 (DGGE-ITS2; cf. 22, 29) identified four clade C types with distribution patterns similar to those previously described for *S. pistillata* (29). Subcladal type C79 occurred only in deep colonies ($n = 7$), types C35/a ($n = 17$) and C78 ($n = 6$) occurred only in shallow colonies, and type C8/a ($n = 16$) was found at both depths.

Bleaching susceptibility. Subcladal symbiont identity had a significant effect ($G = 37.6$, 6; $P < 0.001$) on the response to elevated temperatures (Fig. 2*A*). Visual assessments during March 2006 showed that colonies with symbionts C79 or C35/a were bleached completely white whereas colonies with C78 or C8/a appeared healthy (i.e., no visible changes in colony coloration comparing March/November 2005 with March 2006). Symbiont densities supported the visual assessments where bleached C79 and C35/a colonies lost a greater percentage (≈100%) of *Symbiodinium* cells compared with C78 and C8/a colonies as a result of the increased temperatures in March 2006. Nonetheless, healthy-looking colonies with symbionts C78 and C8/a also showed a large reduction in symbiont numbers as a result of the 2006 thermal anomaly (62–73% loss; Fig. 2*A*).

Recovery. The fine-scale variability in symbiont identity was also tightly linked to the capacity of the entire host colony to recover from bleaching ($G = 23.4$, 6; $P < 0.001$). C78 and C8/a colonies had high total colony survival, whereas individuals harboring types C79 or C35/a suffered severe (>50% of colony) or complete colony mortality (Fig. 2*B*). The capacity to survive adverse conditions therefore differed with survival capacity from high to low with colonies harboring C8/a > C78 > C35/a > C79.

Symbiont shuffling or stability. All C78 and C8/a colonies remained stable throughout the four sampling periods and no additional subcladal types were detected over the two-year monitoring period. In C79 and C35/a colonies additional subcladal types were detected in the ITS2-DGGE profiles during the March 2006 bleaching (Fig. 3, M6). All but one (that showed no change) of the C79 colonies ($n = 6$) showed traces of or became completely dominated by C35/a as well as a previously unidentified C type (1 bp different from C1b). In one of these C79 colonies type C8/a was further detected as a secondary symbiont (Fig. 3). Additional bands characteristic of C78 and C8/a were detected in six C35/a colonies, whereas the remaining 10 colonies showed no changes (Fig. 3). Postbleaching samples of surviving C79 colonies showed a reversion to the characteristic C79 profile, with the exception of one colony that maintained C8/a in addition to C79. Similarly, all C35/a colonies reverted back to their original C35/a profiles, with the exception of two colonies that maintained C78 in addition to C35/a (Fig. 3).

Physiological Data. Photosynthetic performance of the symbionts was measured within the host throughout the sampling period. Seasonal differences of effective quantum yield (ΔFv/Fm′) were only noted in C35/a colonies (November 2006 > March 2006; Table 1), whereas dark-adapted yield (Fv/Fm) showed seasonal differences for all subcladal types except C78 (Table 1). Fv/Fm decreased significantly by 21.3% and 18.5% for C79 and C35/a colonies respectively during bleaching (March 2006) compared with values measured in March 2005 (Table 1). No significant differences were apparent between November 2005 and November 2006 for C35/a, C79, and C78 colonies, but C8/a colonies showed an 8% decrease in Fv/Fm. Comparing symbiont subcladal

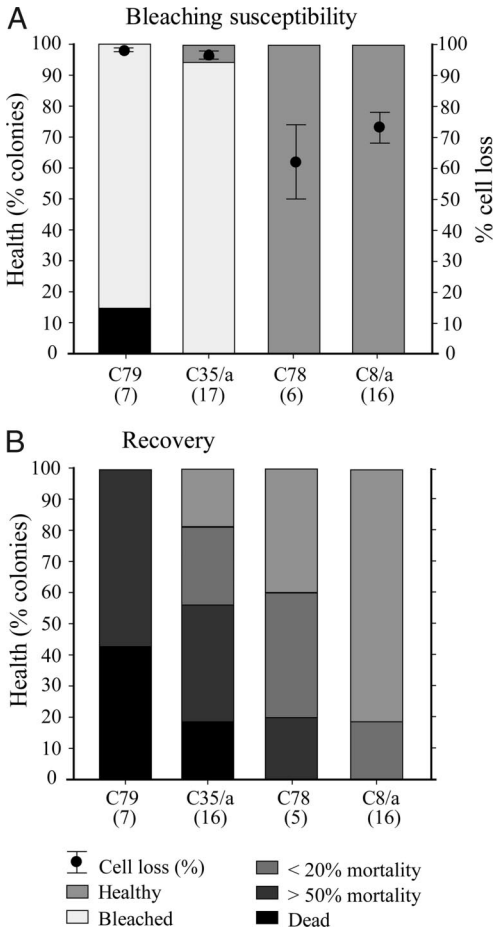


Fig. 2. Bleaching susceptibility and recovery of *Stylophora pistillata* colonies as a function of symbiont type. (*A*) Left axis: Cumulative histogram of the *in situ* visual assessments of bleaching susceptibility of *S. pistillata* colonies as a factor of symbiont type. Right axis: Symbiont cell loss expressed as a percentage loss (± SE) because bleaching by comparing March 2006 (bleached) with March 2005 (normal). (*B*) The recovery potential of *S. pistillata* colonies as a factor of symbiont type based on *in situ* visual assessments 8 months postbleaching (November 2006). Visual assessments were divided into categories: completely healthy, bleached, 10–20% mortality, >50% mortality, or total colony mortality. Number of examined colonies is shown in parentheses below the symbiont types.

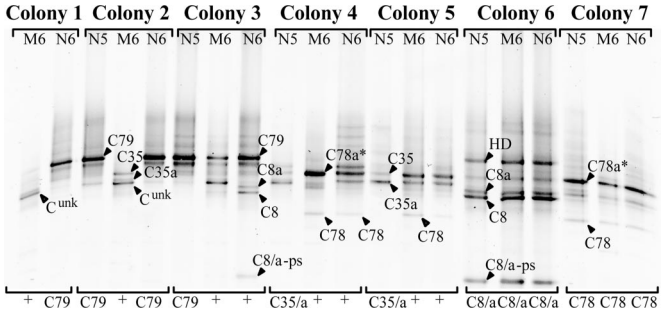


Fig. 3. ITS2-DGGE symbiont profiles of seven individual colonies of *S. pistillata* at three time points: N5 (November 2005, prebleaching), M6 (March 2006, during bleaching), and N6 (November 2006, 8 months postbleaching). March 2005 profiles were identical to November 2005 and are not shown. Symbiont profiles C79 (colonies 1–3) and C35/a (colonies 4 and 5) showed additional bands belonging to symbiont types C8/a, C78, C35/a, or $C1^{unk}$ during bleaching (M6), whereas C8/a and C78 profiles (colonies 6 and 7) remained stable. Representative characteristic bands that were sequenced are marked on the gel, and all sequences belong to known ITS2-DGGE types except $C1^{unk}$. The lowest C8/a band represents a previously identified pseudogene (C8/a-ps) (29) and HD indicates a heteroduplex formed during PCR. Symbiont designation is shown below each lane; + indicates a symbiont is present other than the original. *C78a was wrongly identified as C1 in ref. 29. Sequence C78a contains a 1bp deletion difference from C1 and occurs in conjunction with C78.

types at the same time of the year showed that the bleaching-sensitive C79 and C35/a colonies had lower Fv/Fm and ΔFv/Fm′ values than the more tolerant C78 and C8/a colonies, but differed significantly only when compared with C8/a (Table 1).

Host protein content showed similar patterns to the photosynthetic capacity of the symbionts. Colonies with C79 or C35/a experienced a decrease in host protein of ≈31% as a result of bleaching (March 2006 relative to March 2005; Table 2). Direct comparison of symbiont types during the bleaching time point further showed that C79 or C35/a colonies had significantly lower protein content than colonies containing C78 or C8/a (Table 2).

Discussion

The diverse response of corals to thermal anomalies has been related to host (13, 14, 32) and/or symbiont factors (18, 25, 27, 33) but the combined physiology of the two organisms likely determines the tolerance range of the holobiont (14, 15, 32, 34). The symbionts present in *S. pistillata* on the sGBR have been shown to occupy a specific niche (29) and may confer particular characteristics to their host. This could result in differences in host factors depending on their symbiont and affect stress responses. To fully address this question, more in-depth comparisons of host parameters between different host–symbiont combinations are necessary that were beyond the scope of this study. Nonetheless, the bleaching response of each host–symbiont combination was linked to subcladal genetic differences of the symbiont. This has previously only been documented on the cladal level to explain differences in thermal tolerance between corals (e.g., 16, 17), with those harboring clade D having an increased thermal tolerance over those with clade C (18, 21, 33). However, recent reports also show members of clade D in marginal habitats or cooler high-latitude communities (26, 35–37). These field observations coupled with findings that cultured *Symbiodinium* of the same clade can respond differently to thermal stress indicates that physiological characteristics are not widespread at the cladal level (25, 38).

Here we show that the four subcladal C types (C79, C35/a, C78, and C8/a) in *S. pistillata* are distinctly different in their response to a natural bleaching event. Previous studies position *S. pistillata* symbionts among the most sensitive to experimentally increased levels of temperature and light (14, 39), which is confirmed by field observations during bleaching (9, 10). Our data shows that not all *S. pistillata* colonies are equally affected during bleaching, and colonies with C79 and C35/a are highly sensitive, whereas those with C78 and C8/a are more thermally tolerant. Our results differ from a previous field study that found no correlation between subcladal types and bleaching response (31). However, that study sampled *S. pistillata* colonies 4–5 months after the 2002 bleaching and the occurrence of differential mortality or recovery between colonies with different symbiont types may have caused the apparent lack of correlation (31). By monitoring individual colonies and their symbiont identity before, during, and after bleaching we were able to show that increased sea temperatures resulted in significant bleaching of colonies with C79 and C35/a symbionts, whereas those with C78 and C8/a appeared healthy. Seasonal reductions in symbiont densities form part of natural cycles (40, 41) but prolonged or intensified periods of heating cause extreme reductions of symbiont cells from host tissues (≈ >70%), whereupon normal light levels lead to severe photoinhibition of remaining symbionts (42). This may explain why C78 and C8/a colonies, despite

Table 1. Effective [ΔFv/Fm′, $F(9, 87) = 3.571$, $P = 0.0008$] (±SE) and dark-adapted quantum yield [Fv/Fm, $F(9, 90) = 4.454$, $P < 0.0001$] (±SE) of *Stylophora pistillata* subcladal types (C79, C35/a, C78, or C8/a) measured in March 2005, November 2005, March 2006, and November 2006

Sampling time	C35/a (n = 11, 12)	C79 (n = 4, 4)	C78 (n = 5, 5)	C8/a (n = 13, 13)
March 2005				
ΔFv/Fm′	0.607 ± 0.019	0.606 ± 0.015	0.582 ± 0.045	0.592 ± 0.027
Fv/Fm	0.705 ± 0.005 ***c, *d	0.727 ± 0.007 ***c	0.678 ± 0.011	0.700 ± 0.007 **d
November 2005				
ΔFv/Fm′	0.601 ± 0.022	0.606 ± 0.017	0.583 ± 0.054	0.591 ± 0.026
Fv/Fm	0.668 ± 0.004 ***c	0.658 ± 0.007	0.639 ± 0.014	0.695 ± 0.005 *d
March 2006				
ΔFv/Fm′	0.535 ± 0.051 **d, ##C8/a	0.492 ± 0.081 ##C8/a	0.686 ± 0.026	0.699 ± 0.015 ##C79, ##C35/a
Fv/Fm	0.575 ± 0.024 ***ab, **d, ##C8/a	0.572 ± 0.040 ***a, #C8/a	0.646 ± 0.028	0.657 ± 0.013 #C79, ##C35/a
November 2006				
ΔFv/Fm′	0.685 ± 0.015 **c	0.589 ± 0.023	0.681 ± 0.014	0.687 ± 0.015
Fv/Fm	0.645 ± 0.010 *a, **c	0.659 ± 0.012	0.635 ± 0.007	0.639 ± 0.010 *b, **a

Statistical analysis showed interactive effects of time and symbiont type; post hoc (Tukey LSD) results are shown only where significant. Seasonal differences within a single symbiont are indicated as: *, $0.01 < P < 0.05$; **, $0.001 < P < 0.01$; ***, $P < 0.001$ in relation to time points March 2005, November 2005, March 2006, and November 2006. Significant differences between symbiont types at the same time of year are shown with #, $0.01 < P < 0.05$; ##, $0.001 < P < 0.01$; ###, $P < 0.001$ followed by the respective significantly different symbiont type.

losing ≈60–70% of their symbionts, were not severely affected by bleaching. The symbiont loss experienced by C79 and C35/a colonies (≈100%) was extreme and resulted in a significant reduction of both effective- and dark-adapted yield, suggesting permanent damage to the photosystems (41, 43). As a result, C79 and C35/a colonies likely experienced starvation because they had reduced protein levels during bleaching. This undoubtedly influenced postbleaching survival (Fig. 2*B*) where C79 and C35/a colonies experienced higher mortality rates compared with C78 and C8/a colonies.

Much debate has arisen on the capacity of different symbionts to allow for the rapid adaptation of the holobiont in response to environmental change. Although the adaptive bleaching hypothesis (ABH) has found support in a number of studies (19, 21, 44), increasing evidence now points toward a highly specific and persistent nature of established host–symbiont associations (24, 45–47). This is supported by our data whereby symbiont communities in *S. pistillata* were stable over two years despite minor fluctuations observed during bleaching. No additional subcladal types were detected in C78 and C8/a colonies, but four additional subcladal types were found in C79 and C35/a colonies during bleaching. Despite the recent finding of background levels of clade D in *S. pistillata* (44), all of the additional subcladal types detected belonged to clade C. The majority of these subcladal types (C78, C8/a, and C35/a) are documented to naturally occur within *S. pistillata* populations (this study; 29), but a previously undocumented C type that is closely related to pocilloporid type C1b was also found. Based on the ABH it seems intuitive that the retention of the more thermally resistant types would be beneficial and provide increased resistance to future thermal stress events, but the presence of the more thermally tolerant C78 and C8/a types in ≈30% of thermally sensitive C35/a colonies did not increase postbleaching survival. Furthermore, in all but two colonies complete postbleaching reversion to the original symbiont type was observed. This occurred relatively rapidly compared with other studies (21, 47) suggesting that shifts between closely related subcladal types are perhaps easier than between cladal types. Based on our data, it is difficult to determine whether the altered symbiont communities during bleaching originated from newly acquired symbionts or from background populations present within the host tissues. However, because these symbionts mainly belonged to those naturally present within the host populations, we believe that the rapid changes in symbiont communities proposed by the ABH may be restricted to symbiont types native to the host. Even within this framework it appears that changes in symbiont communities are not sustained postbleaching, and permanent changes are more likely to occur over generations rather than within the life cycle of individual hosts (24, 45–48).

Given the projected influence and effects of climate-driven changes on coral reef ecosystems, information on the mechanism by which symbiont communities change is important because it will affect how coral reef communities respond. Studies that report on symbiont community shifts generally conclude in favor of the ABH, whereby symbionts switch or shift as a result of bleaching, thereby providing hope of increased resilience of coral communities to future thermal stress (18, 19, 21, 31, 44). Most of these studies duly acknowledge that the lack of prebleaching knowledge of the symbiont identities prevents them to account for differential colony mortality. The present *S. pistillata* data, whereby tagged individual colonies were monitored over time, provided the ideal opportunity to address the fundamental question

Table 2. Average soluble protein content [mg·cm^{-2}; $F(9, 96) = 3.272$, $P = 0.0023$] of *Stylophora pistillata* colonies measured in March 2005, November 2005, March 2006, and November 2006

Sampling time	C35/a (n = 12)	C79 (n = 4)	C78 (n = 4)	C8a (n = 7)
March 2005	0.702 ± 0.061	0.527 ± 0.070	0.739 ± 0.030	0.570 ± 0.070
November 2005	0.878 ± 0.063 ***c	0.541 ± 0.052 #C78	1.066 ± 0.136 #C79	0.782 ± 0.059
March 2006	0.480 ± 0.065 ***c, #C8/a, ###C78	0.362 ± 0.046 #C8/a, ###C78	1.150 ± 0.144 ###C35/a, ##C79	0.806 ± 0.073 #C79, #C35/a
November 2006	0.680 ± 0.063	0.455 ± 0.081	0.865 ± 0.072	0.725 ± 0.058

Statistical analysis showed interactive effects of time and symbiont type; post hoc (Tukey LSD) results are shown only where significant. Seasonal differences within a single symbiont are indicated as: *, $0.01 < P < 0.05$; **, $0.001 < P < 0.01$; ***, $P < 0.001$ in relation to time points March 2005, November 2005, March 2006, and November 2006. Significant differences between symbiont types at the same time of year are shown with #, $0.01 < P < 0.05$; ##, $0.001 < P < 0.01$; ###, $P < 0.001$ followed by the respective significantly different symbiont type.

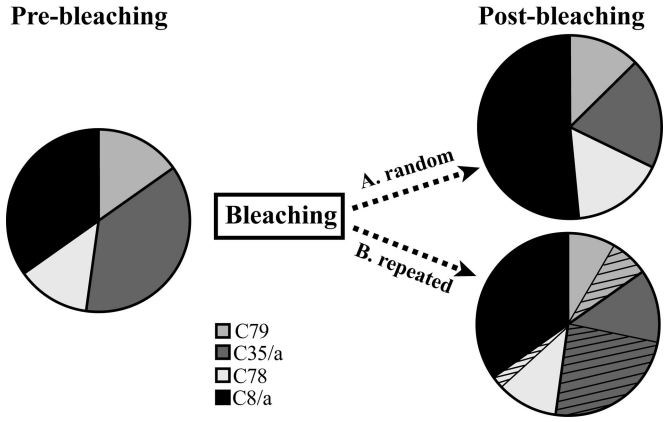


Fig. 4. Changes in the symbiont community structure in *Stylophora pistillata* based on random versus repeated sampling methodologies. (*A*) Random assessments of postbleaching community structure do not account for symbiont-specific colony mortality and subsequently show a marked change in the relative proportions of symbionts present on the reef. (*B*) Repeated sampling highlights the importance of symbiont-specific differential colony mortality as a result of bleaching. The hatched lines represent the relative abundance of dead colonies within each symbiont type. Comparing mechanisms of path A versus B highlights that shifts in the symbiont community are driven by differential mortality as opposed to sustained changes in symbiont types of individual colonies.

of how symbiont displacement versus differential mortality affects community change.

Having both pre- and postbleaching information, we place our data into a framework where pre- and postbleaching symbiont identity is not linked to individual colonies but rather assumes a random sampling strategy as done in previous studies favoring symbiont shuffling/switching (19, 21, 31) (Fig. 4*A*). By using a random sampling strategy, an ≈18% increase of the more thermally tolerant symbiont types (C78 and C8/a) was detected postbleaching. This result bears striking similarity to reported reef wide increases in the abundance of clade D postbleaching based on random pre- and postbleaching sampling (19). Comparing results from a random sampling strategy with those from a repeated sampling approach (Fig. 4*B*) highlights a clear incongruity, with the repeated sampling method providing a more accurate representation of the mode of community change. This latter approach illustrates that symbiont community changes on coral reefs may be more accurately explained by differential mortality as opposed to other reported mechanisms. That said, we acknowledge that repeated sampling of colonies is a difficult task in large-scale sampling efforts that aim to cover multiple taxa and geographic locations; however, conclusions and future interpretations on symbiont community change that are based on random sampling strategies should be approached with caution.

In conclusion, besides illustrating that broad cladal designations may not be sufficient to describe differences in bleaching susceptibility, the data highlight the importance of establishing prebleaching information on an individual basis. We show that community-wide shifts in symbiont dominance are caused by differential mortality of colonies harboring sensitive versus tolerant symbiont types rather than symbiont changes within individual colonies. The relative increase in thermally tolerant symbionts, because of the increased mortality of colonies harboring sensitive types, will have significant long-term implications for coral reef communities. This mechanism is fundamentally different from that proposed by the ABH where shuffling/switching of symbionts occurs in response to adverse environmental conditions and allows individual colonies to adopt more resistant symbionts. Although the *Symbiodinium* genetic diversity may be reduced, there is no concomitant reduction in coral host genetic diversity. In contrast, differential mortality results in a reduction of both the *Symbiodinium* and the coral host genetic diversity on the reef. This difference is fundamental to our understanding of coral reef community change whereby, despite the fact that more thermally tolerant individuals will occur within the population, the overall loss of biodiversity of both symbiotic partners will negatively impact the resilience as well as the ecosystem functioning of coral reefs.

Materials and Methods

In March 2005, colonies of *S. pistillata* were tagged at two depths, 3–6 m (n = 34) and 15–18 m (n = 12), at Heron Island (sGBR). Colonies (>30 cm diameter) were selected to allow for repeated collections over multiple sampling times. Measurements and samples for genetic and physiological analysis were taken in March 2005, November 2005, March 2006, and November 2006, thereby covering two years that included a natural bleaching event in March 2006. Colony health was visually assessed *in situ* and a photographic reference was taken for each colony at each time point. Unfortunately, not all colonies could be sampled at each time point because of logistic problems and numbers used in each analysis are shown in the text or figures.

Physical Data. Water temperature and irradiance levels were measured at both depths throughout the entire period by using loggers (Dataflow, Odyssey) set at logging intervals of 30 min. Winter and summer temperatures were calculated by using the daily averages, maximum and minimum from mid-June to mid-September and mid-December to mid-February, respectively. To assess the effect of irradiance on bleaching, colonies were categorized into one of three groups: deep, shallow-cryptic (reduced direct irradiance due to reef structure), or shallow-exposed colonies (full direct irradiance). Irradiance data were obtained from site averages at the two collection depths as well as a K_d curve that included light directionality (for details, see ref. 49).

Genetic Data. Colony fragments (≈4 cm^2) were collected from the uppermost parts of individual colonies in the morning and placed in shaded flow through aquaria that simulated light levels of the collection site. After the physiological measurements (see below), the coral tissue was removed from each fragment by using an airgun connected to a scuba cylinder. The tissue slurry was diluted in 10 ml of filtered seawater (FSW; 20 μm), and centrifuged at 6,000 × g for 10 min. The supernatant was removed and kept at 4°C for measurement of total host soluble proteins within 24 h (see *Physiological Data*). The remaining algal pellet was resuspended in 5 ml of FSW and homogenized; 0.5 ml was removed for symbiont cell density counts (see *Physiological Data*). The remaining sample was centrifuged for 5 min at 8,000 × g, the supernatant discarded, and the pellet preserved in 20% DMSO buffer (50) and stored at −20°C.

Before DNA extraction the preservation buffer was removed by using a 3-fold DNAB washing step, and the DNA extracted by using a DNeasy Plant kit (Qiagen) according to the manufacturers' instructions (29). The ITS2 region of the ribosomal DNA was amplified by using the primers ITSintfor and ITS2clamp (22) and Platinum TaqDNA-polymerase (Invitrogen) (PCR conditions; cf. 29). Amplified ITS2 fragments were separated by using 30–60% DGGE gels (DCode system, Bio-Rad) during a 14-h run at 100V, after which gels were stained with SYBR green (Invitrogen). Profiles were compared with symbiont profiles previously identified in *S. pistillata* (29), and dominant bands as well as additional bands that appeared during bleaching were excised from each characteristic profile. Bands were eluted in 500 μl of dH_2O, kept overnight at 4°C, and reamplified the next day in a 50-μl PCR with the primers ITSintfor and ITS2-reverse. Successful amplifications were purified by using a PCR-purification kit (Molecular Biology Laboratories) (22, 29) and sequenced at the Australian Genome Research Facility by using the forward and reverse primers in separate reactions. Sequences were identified by a BLAST search on GenBank (www.ncbi.nih.gov) and aligned to previously established ITS2 databases of symbionts from Pocilloporid corals (22, 24, 29, 46).

Physiological Data. A single fragment from each sampled colony at each time point was used for the physiological measurements (see *Genetic Data*). By using pulse amplitude modulated fluorometry (Diving-PAM, WALZ), the photosynthetic efficiency of photosystem II was measured in duplicate (different branch of the same fragment ≈1 cm below the tip) on the collection day at noon (12:00 to 13:00) to obtain the effective quantum yield (ΔFv/Fm′) and again ≈4 h after sunset (22:00 to 23:00) to measure the dark-adapted yield (Fv/Fm) (27, 28). *Symbiodinium* cell densities were counted by using a hemocytometer (Neubauer) with 8–10 replicates of each sample. Finally, protein

concentration (water-soluble) of the host was measured in a UV spectrophotometer (at 280 and 235 nm) (51). Symbiont cell density and protein content were expressed per surface area obtained by the wax weight method (52).

Statistical Analysis. Seawater temperature differences between deep and shallow reef areas were tested by using a two-tailed paired *t* test on all days with data available for both depths. Observational data on health and recovery were tested for distributional effects of symbiont type by using a G-test (Pop-Tools v2.7). The effect of depth on physiological measurements was tested in colonies with symbiont C8/a, but no effect was present and as such all remaining comparative analyses were done using symbiont type and time as factors. Significant interactive effects of time and symbiont type were detected by using repeated measures ANOVAs (STATISTICA v7.1) for the physiological measurements of effective yield, maximum yield and host protein content. Post hoc significance was assessed by using a Tukey-LSD test and results are shown only for biologically relevant comparisons of differences between symbiont types at the same time of year or within a single symbiont type during different times of the year (seasonality).

ACKNOWLEDGMENTS. We thank P. Kaniewska (field assistance), Prof. J-M Hero (statistical advice), and three anonymous reviewers who greatly improved the manuscript. This work was supported by The Ocean Conservancy/International Society for Reef Studies, PADI Foundation, and Project Aware grants (to E.S.) as well as by the Australian Research Council Center of Excellence for Coral Reef Studies and the Coral Reef Targeted Research and Capacity Building for Management Program (O.H.G.).

1. Pandolfi JM, *et al.* (2003) Global trajectories of the long-term decline of coral reef ecosystems. *Science* 301:955–957.
2. Hughes TP, *et al.* (2003) Climate change, human impacts and the resilience of coral reefs. *Science* 301:929–933.
3. Wilkinson CR (1999) Global and local threats to coral reef functioning and existence: review and predictions. *Mar Freshw Res* 50(8):867–878.
4. Bellwood DR, Hughes TP, Folke C, Nyström M (2004) Confronting the coral reef crisis. *Nature* 429:827–833.
5. Donner SD, Skirving WJ, Little CM, 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.
6. Hoegh-Guldberg O, *et al.* (2007) Coral reefs under rapid climate change and ocean acidification. *Science* 318:1737–1742.
7. Hoegh-Guldberg O (1999) Climate change, coral bleaching and the future of the world's coral reefs. *Mar Freshw Res* 50:839–866.
8. Orr JC, *et al.* (2005) Antropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature* 437:681–686.
9. Marshall P, Baird AH (2000) Bleaching of corals on the Great Barrier Reef: Differential susceptibilities among taxa. *Coral Reefs* 19:155–163.
10. Loya Y, *et al.* (2001) Coral bleaching: The winners and the losers. *Ecol Lett* 4:122–131.
11. Edmunds PJ (1994) Evidence that reef-wide patterns of coral bleaching may be the result of the distribution of bleaching susceptible clones. *Mar Biol* 121:137–142.
12. Glynn PW, Mate JL, Baker AC, Calderon MO (2001) Coral bleaching and mortality in Panama and Ecuador during the 1997–1998 El Nino-Southern oscillation event: Spatial/temporal patterns and comparisons with the 1982–1983 event. *Bull Mar Sci* 69:79–109.
13. Brown BE, Dunne RP, Goodson MS, Douglas AE (2002) Experience shapes the susceptibility of a reef coral to bleaching. *Coral Reefs* 21:119–126.
14. Bhagooli R, Hidaka M (2003) Comparison of stress susceptibility of *in hospite* and isolated zooxanthellae among five coral species. *J Exp Mar Biol Ecol* 291:181–197.
15. Visram S, Douglas AE (2007) Resilience and acclimation to bleaching stressors in the scleractinian coral *Porites cylindrical. J Exp Mar Biol Ecol* 349:35–44.
16. Rowan R, Knowlton N (1995) Intraspecific diversity and ecological zonation in coral algal symbiosis. *Proc Natl Acad Sci USA* 92:2850–2853.
17. Rowan R, Knowlton N, Baker A, Jara J (1997) Landscape ecology of algal symbionts creates variation in episodes of coral bleaching. *Nature* 388:265–269.
18. Rowan R (2004) Coral bleaching—Thermal adaptation in reef coral symbionts. *Nature* 430:742.
19. Baker AC, Starger CJ, McClanahan TR, Glynn PW (2004) Corals' adaptive response to climate change. *Nature* 430:741.
20. Fabricius KE, Mieog JC, Colin PL, Idip D, van Oppen MJH (2004) Identity and diversity of coral endosymbionts (zooxanthellae) from three Palauan reefs with contrasting bleaching, temperature and shading histories. *Mol Ecol* 12:2445–2458.
21. Berkelmans R, van Oppen MJH (2006) The role of zooxanthellae in the thermal tolerance of corals: A 'nugget of hope' for corals reefs in an era of climate change. *Proc R Soc Lond Ser B* 273:2305–2312.
22. LaJeunesse TC, *et al.* (2003) Low symbiont diversity in southern Great Barrier Reef corals, relative to those of the Caribbean. *Limnol Oceanogr* 48:2046–2054.
23. LaJeunesse TC, *et al.* (2004) Closely related *Symbiodinium* spp. differ in relative dominance in coral reef host communities across environmental, latitudinal and biogeographic gradients. *Mar Ecol Progr Ser* 284:147–161.
24. Goulet TL (2006) Most corals may not change their symbionts. *Mar Ecol Progr Ser* 321:1–7.
25. Tchernov D, *et al.* (2004) Membrane lipids of symbiotic algae are diagnostic of sensitivity to thermal bleaching in corals. *Proc Natl Acad Sci USA* 101:13531–13535.
26. van Oppen MJH, Palstra. FP, Piquet AMT, Miller DJ (2001) Patterns of coral-dinoflagellate associations in *Acropora*: Significance of local availability and physiology of *Symbiodinium* strains and host-symbiont selectivity. *Proc R Soc Lond Ser B* 268:1759–1767.
27. Iglesias-Prieto R, Beltran VH, LaJeunesse TC, Reyes-Bonilla H, Thome PE (2004) Different algal symbionts explain the vertical distribution of dominant reef corals in the eastern Pacific. *Proc R Soc Lond Ser B* 271:1757–1763.
28. Warner ME, LaJeunesse TC, Robison JE, Thur RM (2006) The ecological distribution and comparative photobiology of symbiotic dinoflagellates from reef corals in Belize: Potential implications for coral bleaching. *Limnol Oceanogr* 51:1887–1897.
29. Sampayo EM, Franceschinis L, Hoegh-Guldberg O, Dove S (2007) Niche partitioning of closely related symbiotic dinoflagellates. *Mol Ecol* 16:3721–3733.
30. Buddemeier RW, Fautin DG (1993) Coral bleaching as an adaptive mechanism - A testable hypothesis. *Bioscience* 43:320–326.
31. van Oppen MJH, Mahiny AJ, Done TJ (2005) Geographic distribution of zooxanthella types in three coral species on the Great Barrier Reef sampled after the 2002 bleaching event. *Coral Reefs* 24:482–487.
32. Goulet TL, Cook CB, Goulet D (2005) Effect of short-term exposure to elevated temperatures and light levels on photosynthesis of different host-symbiont combinations in the *Aiptasia pallida*/*Symbiodinium* symbiosis. *Limnol Oceanogr* 50:1490–1498.
33. LaJeunesse TC, Reyes-Bonilla H, Warner ME (2007) Spring bleaching among ''*Pocillopora*'' in the Sea of Cortez. *Eastern Pacific Coral Reefs* 26:265–270.
34. Iglesias-Prieto R, Trench RK (1997) Photoadaptation, photoacclimation and niche diversification in invertebrate-dinoflagellate symbiosis. *Proc 8th Int Coral Reef Symp* 2:1319–1324.
35. Toller WW, Rowan R, Knowlton N (2001) Zooxanthellae of the *Montastraea annularis* species complex: Patterns of distribution of four taxa of *Symbiodinium* on different reefs and across depths. *Biol Bull* 201:348–359.
36. Chen CA, Wang J-T, Fang L-S, Yang Y-W (2005) Fluctuating algal symbiont communities in *Acropora palifera* (Scleractinia: Acroporidae) from Taiwan. *Mar Ecol Progr Ser* 295:113–121.
37. Lien Y-T, *et al.* (2007) Occurrence of the putatively heat-tolerant *Symbiodinium* phylotype D in high-latitudinal outlying coral communities. *Coral Reefs* 26:35–44.
38. Robison JD, Warner ME (2006) Differential impacts of photoacclimation and thermal stress on the photobiology of four different phylotypes of *Symbiodinium* (Pyrrhophyta). *J Phycol* 42:568–579.
39. Bhagooli R, Hidaka M (2004) Photoinhibition, bleaching susceptibility and mortality in two scleractinian corals, *Platygyra ryukyuensis* and *Stylophora pistillata* in response to thermal and light stresses. *Comp Biochem Physiol A* 137:547–555.
40. Fitt WK, McFarland FK, Warner ME, Chilcoat GC (2000) Seasonal patterns of tissue biomass and densities of symbiotic dinoflagellates in reef corals and relation to coral bleaching. *Limnol Oceanogr* 45:677–685.
41. Warner ME, Chilcoat GC, McFarland FK, Fitt WK (2002) Seasonal fluctuations in the photosynthetic capacity of photosystem II in symbiotic dinoflagellates in the Caribbean reef building coral *Montastraea. Mar Biol* 141:31–38.
42. Enriquez S, Mendez ER, Iglesias-Prieto R (2005) Multiple scattering on coral skeletons enhances light absorption by symbiotic algae. *Limnol Oceanogr* 50:1025–1032.
43. Jones RJ, Hoegh-Guldberg O (2001) Diurnal changes in the photochemical efficiency of the symbiotic dinoflagellates (Dinophyceae) of corals: Photoprotection, photoinactivation and the relationship to coral bleaching. *Plant Cell Env* 24:89–99.
44. Mieog JC, van Oppen MJH, Cantin NE, Stam WE, Olsen LJ (2007) Real-time PCR reveals a high incidence of *Symbiodinium* clade D at low levels in four scleractinian corals across the Great Barrier Reef: implications for symbiont shuffling. *Coral Reefs* 26:449–457.
45. Goulet TL, Coffroth MA (2003) Genetic composition of zooxanthellae between and within colonies of the octocoral *Plexaura kuna*, based on small subunit rDNA and multilocus DNA fingerprinting. *Mar Biol* 142:233–239.
46. LaJeunesse TC (2005) Species radiations of symbiotic dinoflagellates in the Atlantic and Indo-Pacific since the Miocene-Pliocene transition. *Mol Biol Evol* 22:570–581.
47. Thornhill D, LaJeunesse TC, Kemp DW, Fitt WKW, Schmidt GW (2006) Multi-year seasonal genotypic surveys of coral-algal symbiosis reveal prevalent stability or post-bleaching reversion. *Mar Biol* 148:711–722.
48. Baird AH, Cumbo VR, Leggat W, Rodriguez-Lanetty M (2007) Fidelity and flexibility in coral symbiosis. *Mar Ecol Prog Ser* 347:307–309.
49. Sampayo EM (2007) Ecology and diversity of *Symbiodinium* in Pocilloporid corals. PhD thesis (University of Queensland, St. Lucia, Australia).
50. Seutin G, White BN, Boag PT (1991) Preservation of avian blood and tissue samples for DNA analyses. *Can J Zool* 69:82–90.
51. Whitaker JR, Granum PE (1980) An absolute method for protein determination based on difference in absorbance at 235 and 280 nm. *Anal Biochem* 109:156–159.
52. Stimson J, Kinzie RA (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.