ECOLOGY

What makes a winner? Symbiont and host dynamics determine Caribbean octocoral resilience to bleaching

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Unlike reef-building, scleractinian corals, Caribbean soft corals (octocorals) have not suffered marked declines in abundance associated with anthropogenic ocean warming. Both octocorals and reef-building scleractinians depend on a nutritional symbiosis with single-celled algae living within their tissues. In both groups, increased ocean temperatures can induce symbiont loss (bleaching) and coral death. Multiple heat waves from 2014 to 2016 resulted in widespread damage to reef ecosystems and provided an opportunity to examine the bleaching response of three Caribbean octocoral species. Symbiont densities declined during the heat waves but recovered quickly, and colony mortality was low. The dominant symbiont genotypes within a host generally did not change, and all colonies hosted symbiont species in the genus *Breviolum*. Their association with thermally tolerant symbionts likely contributes to the octocoral holobiont's resistance to mortality and the resilience of their symbiont populations. The resistance and resilience of Caribbean octocorals offer clues for the future of coral reefs.

INTRODUCTION

Reef corals, which create the structural framework for some of the most biologically diverse (*1*) and economically important (*2*) ecosystems on the planet, are declining (*3*). This decline is due to a range of anthropogenic and natural perturbations, the foremost being increased ocean temperatures (*4*). Increased sea surface temperatures can lead to a breakdown of the nutritional mutualism between reef cnidarians and endosymbiotic algae in the family Symbiodiniaceae (*4*). Many cnidarians, including reef-building stony corals (scleractinians) and soft corals (octocorals), depend on their algal endosymbionts for nutrients and growth (*5*) and the loss of these algal symbionts, termed coral bleaching, can result in coral death.

Over the past four decades, coral bleaching has become increasingly common and is now arguably the most important determinant of coral reef ecosystem dynamics. Most studies of bleaching have focused on scleractinians, as these ecosystem engineers play a crucial role in determining reef structure and have seen major bleaching-related population declines. Studies have shown that variation in scleractinian bleaching response is due in part to the genetic identity of the symbiont, with scleractinians that do not bleach often harboring thermally tolerant symbionts, such as *Cladocopium thermophilum*, or species within the genus *Durusdinium* (*6*–*9*). In contrast to their scleractinian relatives, Caribbean octocorals have not exhibited population declines in recent years; rather, these anthozoans are maintaining coral cover and at some sites, increasing in abundance despite rising oceanic heat content (*10*–*12*). The basis for the comparative success of this group in the face of anthropogenic ocean warming remains unclear, however.

The ability of organisms to persist in the face of a stressor can be due to resistance (the ability to withstand disturbance) or resilience (the ability to recover after disturbance). Historically, bleaching among Caribbean octocorals has been rare, particularly in comparison to scleractinians (*13*–*18*), suggesting that these holobionts are likely exhibiting resistance, either at the colony or symbiont level (*17*). Notably, symbiont diversity among Caribbean octocorals is lower than that of scleractinian species with the vast majority of Caribbean octocorals harboring symbionts in the *Breviolum* B1/B184 lineage [nomenclature based on variation in the *internal transcribed spacer region 2* (*ITS2*) of *nuclear ribosomal DNA* (*rDNA*) and the *chloroplast 23S rDNA* (cp-*23S rDNA*), respectively (*19*–*21*)]. This association with symbiont species in the genus *Breviolum* may contribute to octocorals' resistance to bleaching, as there is considerable genetic diversity within this lineage (*22*–*24*) and laboratory studies have shown that several of these symbiont species grow well at elevated temperatures (*25*–*27*). Furthermore, in the few reports of Caribbean octocoral bleaching before 2000 [1983 (*13*), 1987 (*28*), and 1998 (*29*)], bleaching was generally restricted to octocoral hosts that did not typically harbor *Breviolum* B1 symbiont species [i.e., *Plexaurella dichotoma* (*20*, *21*), *Plexaurella nutans* (*21*), *Eunicea* sp. (*19*), *Erythropodium caribaeorum* (*19*, *20*), and *Briareum asbestinum* (*15*)]. Thus, one possible explanation for the continued success of Caribbean octocorals is the prevalence of symbioses with symbiont species within the *Breviolum* B1 lineage, with bleaching-resistant hosts harboring more thermally tolerant symbiont species or genotypes. Yet, resilience is also likely to play a key role in the stability of octocoral populations. In addition to harboring potentially thermal tolerant symbionts, bleaching-resistant and -resilient octocorals may be able to shuffle symbiont types in response to thermal stress. This latter scenario is supported by observations of bleaching within *B. asbestinum*. When the octocoral *B. asbestinum* bleaches, the dominant symbiont, a species in the *Breviolum* B19 lineage, is replaced by a more thermally tolerant species in the *Breviolum* B1

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lineage (*15*, *30*, *31*). This ability to shift symbiont partners during and after bleaching also suggests a role of resilience in the maintenance of octocoral communities.

With the exception of the *B. asbestinum* studies already noted, the few analyses of symbiont types within bleached Caribbean octocorals have been short-term laboratory-based studies (*17*, *18*, *32*). There have been no field studies tracking the dynamics of bleaching across a marine heat wave among Caribbean octocorals. Symbiont types within octocorals during a bleaching event have been characterized in soft coral species from the Great Barrier Reef (GBR) and Guam (*33*, *34*), but those host species are morphologically, ecologically, and taxonomically distinct from the Caribbean species. Unlike Caribbean species, the majority of studied Pacific octocoral species harbor symbionts within *Cladocopium* or *Durusdinium* (*21*, *33*, *34*). As symbionts vary physiologically in their response to thermal stress both within and between species (*25*–*27*, *35*, *36*), it is important to examine the dynamics of coral-algal symbiosis and its ability to cope with the changing temperature at multiple levels of resolution. As bleaching susceptibility may depend on the response of both the host species (*37*) and the symbiont (*7*), approaches examining responses between and within specific host-symbiont pairs must be used.

Although Caribbean octocoral bleaching has historically been rare, the frequency of widespread coral bleaching events is increasing (*38*–*40*), and octocoral bleaching, although it remains relatively uncommon, is also on the rise. In 2014–2017, an extended El Niño–Southern Oscillation event resulted in scleractinian bleaching on a global scale (*41*). Octocoral bleaching was observed in the Florida Keys during the late summer and autumn of 2014 and 2015. This provided a unique opportunity to examine octocoral response to thermal stress over time in a field-based setting. We surveyed bleaching among octocorals in 2014 and then followed the bleaching status and symbiont genotypes of individual colonies of three Caribbean octocoral species before, during, and after the 2015 bleaching event. We specifically asked (i) whether symbiont types changed during bleaching and recovery and (ii) how bleaching susceptibility, resistance, or resilience varied with symbiont genotype and host species. Octocorals have fared relatively well in the past several decades and their increased relative abundance on Caribbean reefs has been suggested to be a "new normal" (*12*), yet our understanding of their response to bleaching lags behind that of scleractinian corals. An in-depth understanding of the dynamics of octocoral-algal symbioses through bleaching events is important in assessing their continued success as sea surface temperatures continue to rise.

RESULTS

2014 observations

At Alligator Reef, where colonies were tagged in September 2014 and resampled in February 2015, 80% of colonies were bleached or partially bleached in September 2014 (table S1). While colonies of several species that bleached in September 2014, including *Eunicea calyculata*, *Plexaurella grisea*, and *P. nutans*, were fully recovered by February 2015, certain octocorals did not recover. Notably, two *B. asbestinum* colonies died before resampling in February 2015, and the remaining tagged *B. asbestinum* colony was pale and partially bleached but recovering (table S1). All surviving colonies harbored the same symbiont lineage in February 2015 as they initially did in September 2014, except for the singular surviving *B. asbestinum* colony, which switched from harboring *Breviolum* B19 (cp-type B178) to *Breviolum* B1 (cp-type B184; table S1).

Bleaching was widespread at five additional sites surveyed in September 2014 (tables S2 and S3) and included many species that rarely have been observed to bleach (table S2). Symbiont genus assignment, determined for a subset of colonies sampled from the six sites during September 2014, established that all colonies harbored symbionts within the *Breviolum* B1 lineage, except for *B. asbestinum*, which harbored symbionts within the *Breviolum* B19 (cp-type B178) lineage and one *P. nutans* colony, which harbored symbionts within both the *Breviolum* B1 lineage and genus *Cladocopium* (cp-type 180; tables S1 and S2).

2015 sea surface temperature

In May 2015, three octocoral species were tagged and monitored over 15 months for symbiont density and genotype on two patch reefs Soft Coral 2 (SC2; N24 49.478, W 80.41.187) and CoffMacFan (CMF; N24 44.701, W 80 46.806) in the middle Florida Keys (table S4). Both study sites followed similar temperature patterns over the course of the study (Fig. 1). Comparison of the two sites identified significant differences between months (F = 8202.98, df = 27, 188777, $P < 0.001$) and a significant interaction between month and site ($F = 41.26$, $df = 21$, 18777, $P < 0.001$), but no overall difference between sites ($F = 1.627$, $df = 1$, 18777, $P = 0.202$). Inspection of the estimated marginal means showed that SC2 experienced greater temperatures than CMF in May, June, July, and August 2015 and in May, June, and July 2017. CMF experienced greater temperatures than SC2 in October 2015 and January, February, March, May, and December 2016. The pattern of differences between sites is also evident in fig. S1. The highest temperatures were recorded during the summer of 2015, when temperatures averaged 30.3°C at CMF and 30.4°C at SC2 (Fig. 1).

2015 bleaching

For the 3 months before September 2015, daily sea surface temperatures averaged at or above 30°C in the Florida Keys (Fig. 1). Observational data using the CoralWatch coral health chart (*42*) indicated perceptible bleaching in *Muricea atlantica* and *P. dichotoma* during

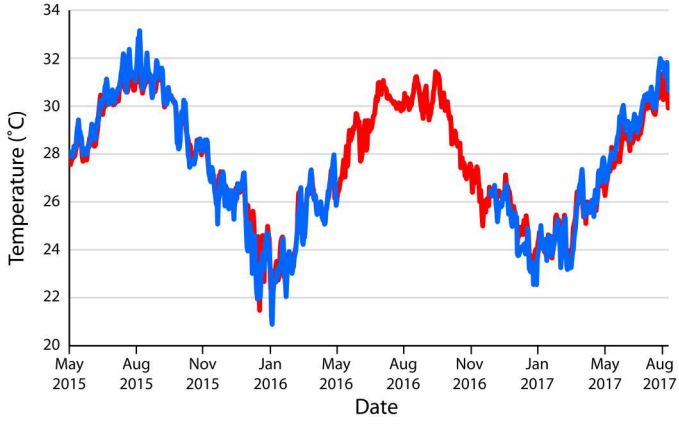

Fig. 1. The temperature at the two sites where octocorals were monitored from May 2015 until August 2017. CMF, red line; SC2, blue line. Note that temperature data are missing at SC2 from May 2016 until December 2016 due to the loss of a temperature logger.

September 2015 and, in most cases, bleaching was homogeneous across the entire colony (Fig. 2). Coloration change in *Muricea elongata* colonies was less obvious over the course of the study. All three species exhibited declines in symbiont densities during this time (Fig. 3), corresponding temporally to the largest degree heating week (DHW) recorded during the study (table S5), and significant differences in cell densities over time [generalized linear mixed model (GENLMM), $F = 64.44$, $df = 7, 93$, $P = 0.001$]. In addition, cell densities differed significantly among host species across the study, with *M. elongata* having greater densities than *M. atlantica* or *P. dichotoma* (GENLMM with a Gamma log-link function, SPSS v28, $F = 8.42$, $df = 2, 453$, $P < 0.001$). There was a significant species-by-time interaction (GENLMM, $F = 13.15$, $df = 14, 101$, $P = 0.001$), reflecting in large part the more muted response of *M. elongata* to the bleaching event. The lowest average symbiont densities occurred in September 2015, followed by the highest average densities during recovery in March 2016 (Fig. 3). The extent of bleaching also differed between species. Symbiont cell density in *M. elongata* colonies decreased by an average of 29% from May 2015 to September 2015, compared to *P. dichotoma* colonies where cell density decreased by an average of 92%. *M. atlantica* colonies were not sampled before the bleaching event, and it is not possible to compare cell densities to a prebleaching record. The lowest symbiont densities in *M. atlantica* were recorded during September 2015 and mirrored cell densities recorded in *P. dichotoma* throughout the remainder of the study (Fig. 3). A significant positive correlation was found between the qualitative CoralWatch bleaching card scores and the quantitative cell count data for each species (*P. dichotoma* adj $R^2 = 0.105$, $P < 0.001$; *M. atlantica* adj $R^2 = 0.276$, $P < 0.001$; *M. elongata*, adj $R^2 = 0.051$, $P < 0.01$; fig. S2).

Symbiont genera

All colonies followed from May 2015 to August 2017 harbored *Breviolum* B1 species as the dominant symbiont type, based on the presence of a single cp-*23S rDNA* fragment size data (cp-type) except as noted below. In addition, many colonies of *P. dichotoma* also harbored symbionts within the genus *Cladocopium* along with *Breviolum* B1 species (Fig. 4). The cp-*23S rDNA* fragment size data (cp-type) were uninformative beyond the level of resolution of genus/subgenus. Phylogenetic analysis from concatenated sequences of an approximately 0.7-kb region of the cp-*23S rDNA* and the approximately 0.25-kb B7Sym15 flanking region separated the symbionts from *M. atlantica*, *M. elongata*, and *P. dichotoma* samples into three monophyletic groups, suggesting that each host species harbored distinct symbiont species within *Breviolum* B1 (Fig. 5).

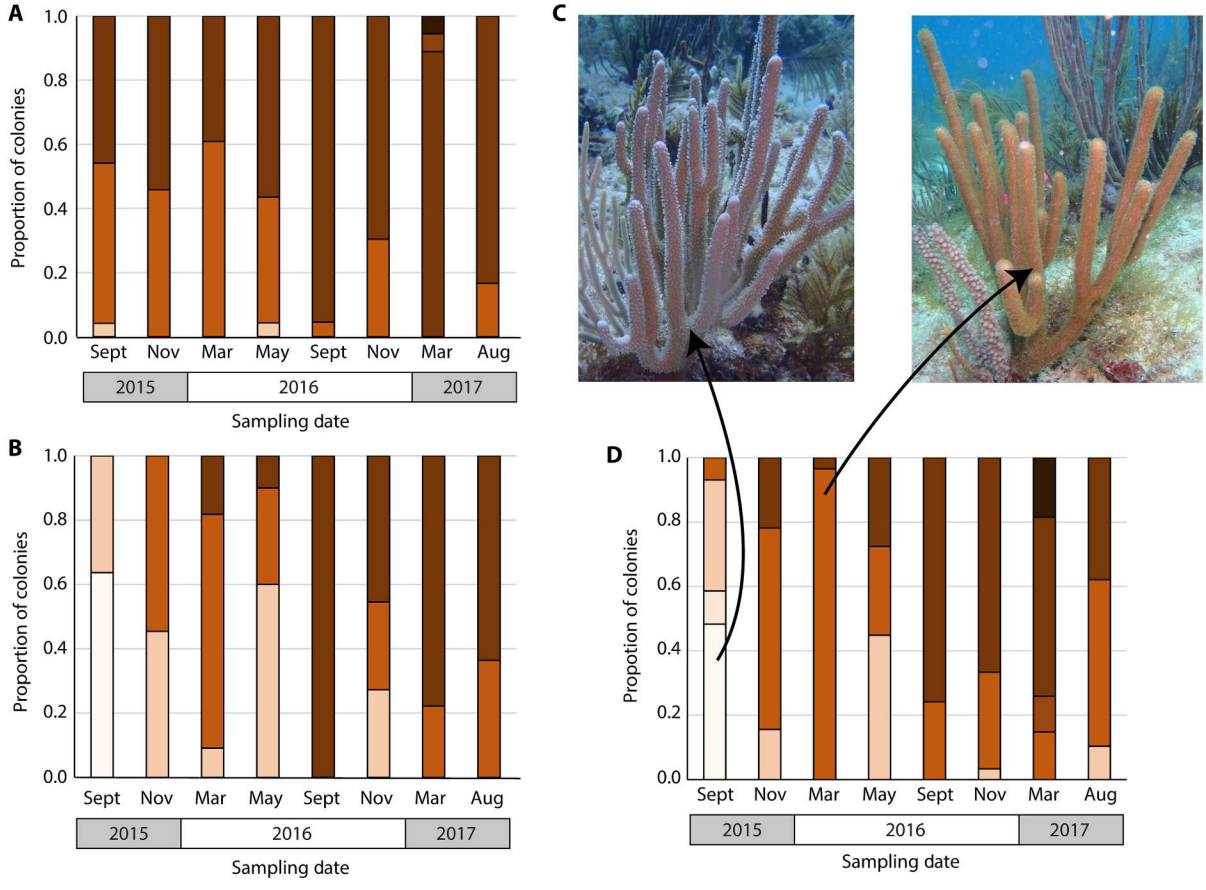

Fig. 2. Bleaching card scores from CoralWatch coral health chart for each species. (**A**) Proportion of *M. elongata* colonies in each bleaching state. (**B**) Proportion of *M. atlantica* colonies in each bleaching state. (**C**) Bleached (left) and recovering (right) *P. dichotoma* with arrows indicating bleaching card score; colony heights, ~30 cm. (**D**) Proportion of *P. dichotoma* colonies in each bleaching state. Colony color was matched to a CoralWatch coral health chart with lighter colors indicating increased bleaching from white (totally bleached) to dark brown (not bleached).

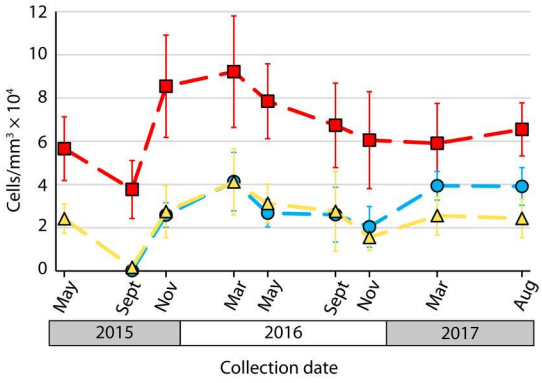

Fig. 3. Symbiont density over time for the three host species from May 2015 to August 2017. *M. atlantica*, blue circle; *M. elongata*, red square; *P. dichotoma*, yellow triangle. Error bars are SD.

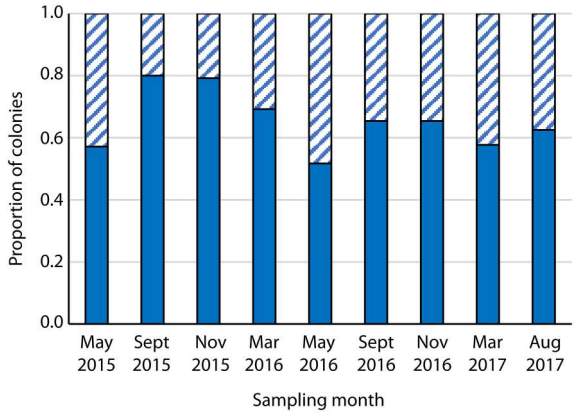

Fig. 4. The proportion of *P. dichotoma* colonies that harbored solely *Breviolum* B1 or both *Breviolum* B1 and *Cladocopium* sp. over the course of the study. Blue, colonies with only *Breviolum* B1 symbionts; blue and white hatching, colonies with both *Breviolum* B1 and *Cladocopium* sp. symbionts.

Symbiont genotypes

Four *Breviolum* microsatellite loci resolved 7, 8, and 37 unique multilocus genotypes (MLGs) from *M. atlantica*, *P. dichotoma*, and *M. elongata* symbionts, respectively. No symbiont genotypes were shared between host species, again supporting the conclusion that each octocoral host species harbored a different species of symbiont.

In one colony each of *M. atlantica* and *P. dichotoma*, we detected multiple symbiont genotypes being harbored simultaneously across four and six sampling times, respectively (figs. S3 and S4 and table S6), indicating that while most colonies of those host species contained only a single, detectable MLG at a given time point, these few colonies regularly harbored multiple symbiont genotypes. In contrast, 15 colonies of *M. elongata* harbored more than one symbiont genotype at one or more sampling times, representing more than 47% of the samples taken from colonies of *M. elongata* (fig. S5 and table S6). However, there was no relationship between allelic diversity (i.e., the number of distinct alleles present in a host colony over the course of the experiment) and cell density (GLMM, $F = 0.03$, $df = 1, 162$, $P = 0.83$) nor with genotypic diversity [i.e., the number of genotypes present ($F = 0.062$, $df = 1, 162$, $P = 0.804$)]. Using allele present-absence data, hierarchical cluster analyses based on Jaccard dissimilarities of the alleles present in each colony for each species identified three clusters in *M. elongata*, two clusters in *M. atlantica*, and two in *P. dichotoma* with two *P. dichotoma* outliers (fig. S6). Cell densities did not differ between the clusters within a host species (general linear model, *M. elongata*, $F = 1.137$, $df = 2, 17$, $P = 0.344$; *M. atlantica*, $F = 1.51$, $df = 2, 6$, $P = 0.294$; *P. dichotoma*, $F = 3.52$, $df = 1, 21$, $P = 0.075$).

Over the course of the study, 36, 56, and 27% colonies of *M. atlantica*, *M. elongata*, and *P. dichotoma*, respectively, changed symbiont genotypes from one time point to the next (figs. S3 to S5 and table S6), with some colonies changing symbiont genotypes multiple times (figs. S3 to S5 and table S6). In many cases, especially among *M. elongata* colonies, changes occurred in colonies that harbored two or more symbiont genotypes simultaneously, with the change involving the gain or loss of one of the genotypes (figs. S3 to S5 and table S6). Changes in genotype were distributed across the study and were not concentrated during the bleaching event of September 2015 (Fig. 6). However, an increase in changes was observed in *M. elongata* in late 2016–2017 ($\chi^2 = 19.739$, $df = 7$, $P = 0.006$). This could represent the appearance of more competitive, potentially less thermal-tolerant genotypes. However, these were all genotypes within the *Breviolum* species harbored by *M. elongata*. We found no pattern of specific genotypes increasing or decreasing in frequency before, during, or after bleaching (Fig. 7). Over the course of the study, low symbiont density (i.e., bleaching) was not associated with any specific MLG (fig. S7).

Mortality

Mortality was generally low but varied over the course of the study and among the species (table S7). We did not observe any mortality during or immediately after the bleaching event (September and November 2015), and there was no mortality of *M. atlantica* colonies for the duration of the study. Two colonies of *M. elongata* and one *P. dichotoma* colony died during the first year, and an additional five colonies of *M. elongata* and two *P. dichotoma* colonies died in the second year of the study (28 and 10% of the colonies for *M. elongata* and one *P. dichotoma*, respectively; table S7). While the mortality rate was greatest among *M. elongata* and lowest in *M. atlantica* (table S7), it did not differ significantly between species ($\chi^2 = 5.794$, $df = 2$, $P = 0.055$). Among *M. elongata* colonies, mortality did not differ with symbiont genotype ($\chi^2 = 0.157$, $df = 1$, $P = 0.69$) nor allelic diversity ($\chi^2 = 3.009$, $df = 2$, $P = 0.22$). Colonies that eventually died did not have significantly different cell densities from survivors at the time of bleaching [September 2015 comparisons, analysis of variance (ANOVA), $P = 0.48$; logistic regression, $P = 0.48$] nor in the interval before their deaths [comparisons with survivors at the time of death, logistic regression at each of the four intervals in which mortality was observed ($P = 0.800$, 0.817, 0.354, and 0.094)].

DISCUSSION

2015 was the warmest year on record at the time of the study and, in contrast to other bleaching events, in both 2014 and 2015, widespread bleaching was reported among octocorals, including species that had rarely bleached in the past (tables S1 and S2). This was evident at our study sites where *M. atlantica* and *P. dichotoma* were visibly bleached in September 2015, and all three host species had significant declines in symbiont density. While bleaching had previously been reported in *P. dichotoma* and possibly *M.*

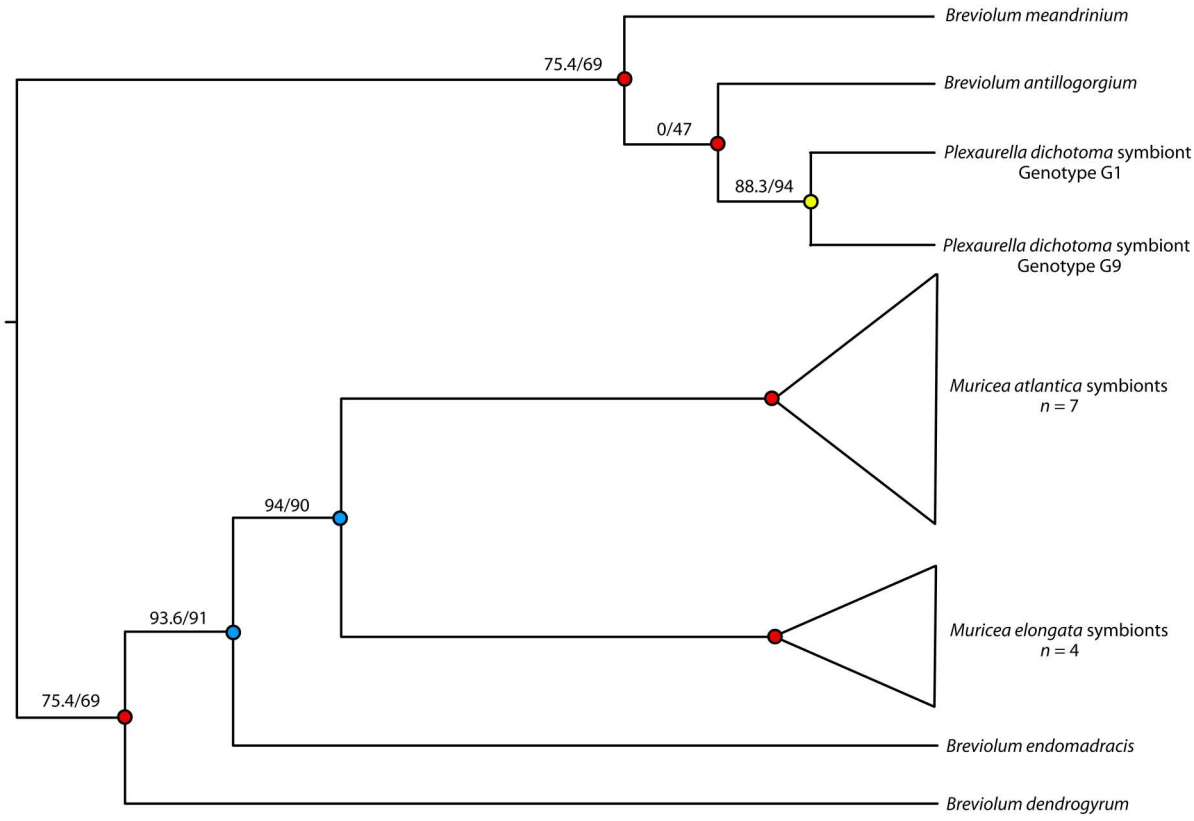

Fig. 5. Maximum likelihood phylogenetic tree of symbionts from the three host species based on sequence variation in domain V of the cp-*23S rDNA* gene and the flanking region of the B7Sym15 microsatellite. Branch lengths have been transformed into a cladogram, and support values (SH-aLRT/ultrafast bootstrap) are shown directly to the left of the node they refer to. Nodes have been colored according to SH-aLRT support values, with >90% support as blue, 85 to 90% as yellow, and <85% as red.

atlantica (*13*, *16*), bleaching appears to be a relatively rare occurrence among most Caribbean octocorals, particularly as compared to bleaching in Caribbean scleractinians.

Despite the loss of symbionts, mortality was low during the marine heat waves. All the tagged colonies survived between the September and November 2015 surveys (i.e., during and immediately following the height of bleaching), and symbiont density in colonies returned to prebleaching levels by November 2015 (Fig. 3). In contrast to the low mortality rates that we observed among *M. atlantica*, Prada *et al.* (*16*) reported high mortality among *Muricea muricata/atlantica* colonies during the 2005 ocean warming event. Our observations of low mortality among colonies of *M. atlantica* suggest that the result reported by Prada *et al.* (*16*) might reflect mortality specific to *M. muricata* or possibly a location-specific mortality. Generally, our observations of low mortality across all studied species are consistent with other studies of Caribbean octocorals (*13*, *16*).

Unlike the widespread bleaching we observed in 2014 and 2015, laboratory studies have found limited sensitivity to high temperatures among Caribbean gorgonians. McCauley *et al.* (*18*) reported relatively minor symbiont loss in *Eunicea tourneforti* and *Pseudoplexaura crucis* ranging from 26 to 35% following short-term (7 days) exposure to elevated temperatures. In a similar study, Goulet *et al.* (*17*) did not observe bleaching in *Eunicea flexuosa*, *E. tourneforti*, and *Pseudoplexaura porosa* after 5 days of temperature stress. The difference between these laboratory studies and our field observations of substantial symbiont loss is likely due to a combination of the sustained exposure to elevated temperatures seen in the field where corals experienced 8 to 13 DHWs, and potentially the host/symbiont species involved.

Several researchers have found that colonies with diverse symbiont populations have lower stress tolerance (*43*) and have proposed that greater diversity within the symbiont community could increase the host's susceptibility to stress. Given that *M. elongata* had high allelic and genotypic diversity (table S6), one might predict greater susceptibility to stress. However, although more *M. elongata* died, the mortality rate was not significantly different from the other host species ($\chi^2 = 5.794$, $df = 2$, $P = 0.055$). In addition, *M. elongata* lost proportionally fewer symbionts during the bleaching event than the other two host species and mortality did not correlate with bleaching susceptibility.

Roles of symbionts and hosts

The coral holobiont is a complex consisting of the coral animal, algal symbionts, and other microbes. In an ecological context, the terms resistance and resilience refer to the responses of a population to a stressor. Resistant populations do not exhibit changes in their vital statistics (e.g., increases in mortality), whereas resilient populations will exhibit such change but then recover to pre-stressor norms. This seemingly simple dichotomy becomes complex when

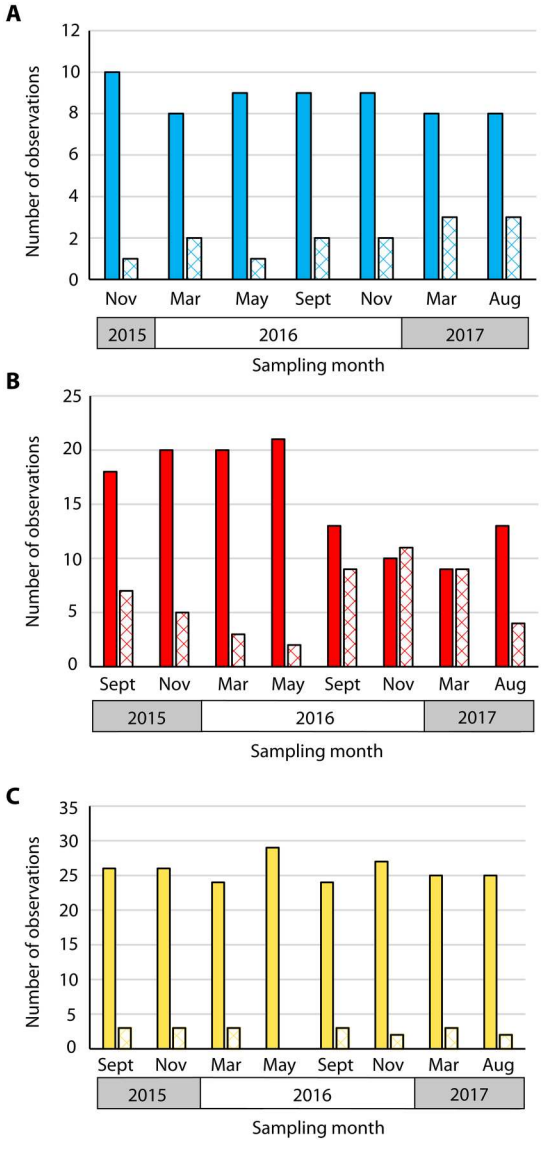

Fig. 6. The number of observations where symbiont genotypes within a colony changed between sampling periods for each species. (**A**) *M. atlantica* (blue), (**B**) *M. elongata* (red), and (**C**) *P. dichotoma* (yellow). Solid bar, no change between sampling periods; Hatched, symbiont genotypes changed between sampling periods.

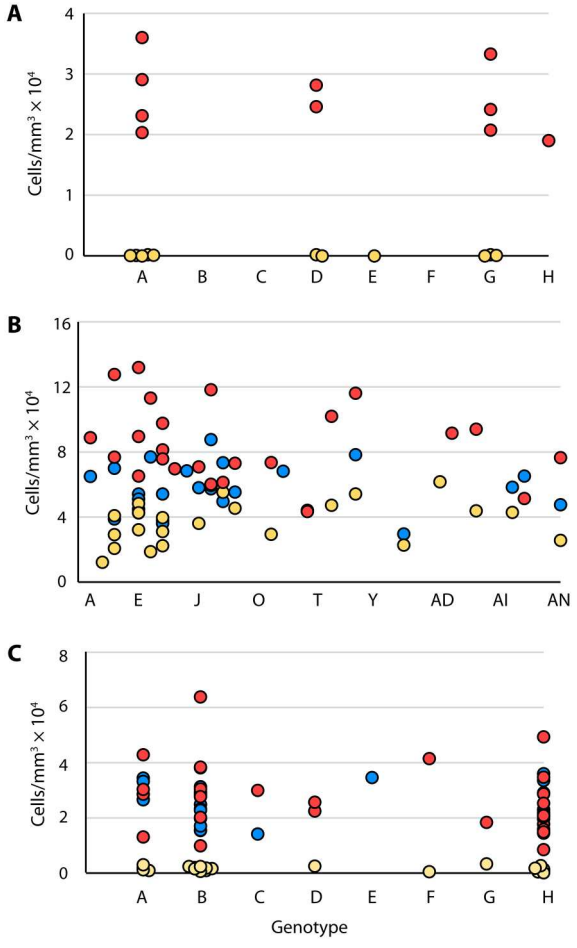

Fig. 7. Cell density by genotype for each species before (May 2015), during (September 2015), and after the bleaching event (November 2015). (**A**) *M. atlantica*, (**B**) *M. elongata*, and (**C**) *P. dichotoma*. Colors: May 2015 (blue), September 2015 (yellow), and November 2015 (red). Note that *M. atlantica* was not monitored in May 2015. Letters on the *x* axis are arbitrary names signifying the MLG designation based on the presence/absence of a given allele for each sample.

we consider organisms as holobionts. In the case of octocorals, there are populations of coral hosts on a reef, and then within each individual host, there are population(s) of symbionts. Using mortality as the measure of resistance, the colonies in our study were strongly resistant to thermal stress in 2014 and 2015. In contrast, the loss of symbionts (i.e., bleaching) suggests a lack of stress resistance at the level of the symbiont populations. However, symbiont populations were resilient as they recovered rapidly. The highest densities were seen in March 2016. That pattern is similar to that observed by Fitt *et al.* (*44*) in *Orbicella* (previously *Montastrea*) *faveolata* and *annularis* after the 1995 bleaching in the Florida Keys. These authors speculated that the overshoot may be the result of increased symbiont growth rates in response to increased nutrient availability due to low symbiont numbers.

Holobiont survival is often attributed to shuffling or switching to a more thermally tolerant symbiont, indicating that symbiont genotype likely plays a role in the holobiont's resistance and/or resilience to thermal stress. Symbiont shuffling in response to elevated temperature (examined at the genus or species level) has been reported among scleractinians (*6*, *8*), but changes in symbiont type are not always seen in response to thermal stress in octocorals and scleractinians [see, e.g., (*17*, *18*, *32*, *45*–*47*)].

Shuffling or switching of symbionts did not account for the holobiont's resistance to thermal stress in *M. atlantica* and *M. elongata* but may have played a role in the response of *P. dichotoma*. Among the *P. dichotoma* colonies, 43% harbored symbionts within the genus *Cladocopium* in May 2015, in addition to the *Breviolum* B1 lineage (Fig. 4). In September 2015, all colonies continued to harbor symbionts within *Breviolum* B1, but the proportion also harboring symbionts within the genus *Cladocopium* had dropped to 20% (Fig. 4). *Cladocopium* populations apparently recovered by May

2016, with 48% of the colonies harboring *Cladocopium* in addition to *Breviolum* (Fig. 4). Thus, *Breviolum* and *Cladocopium* populations differed in their resistance to the thermal stressor, but both were resilient, recovering to prebleaching levels by May 2016. Apart from the loss of symbionts within *Cladocopium* in some colonies of *P. dichotoma*, symbiont genera and species did not change over the course of 27 months of monitoring. This stability extended to the level of the symbiont genotype as most colonies maintained the same *Breviolum* symbiont genotypes across the 27 months (figs. S3 to S5 and table S6). Changes in symbiont genotype observed were not specific to a certain month or sampling point, suggesting that the changes were not a response to bleaching (Fig. 6 and figs. S3 to S5).

Other studies that have examined symbiont composition at the level of intraspecific genetic variation over the course of bleaching have also found stability within host colonies. Goulet and Coffroth (*45*) used DNA fingerprinting to assess genetic identity and found no change in symbiont DNA fingerprints within octocoral clones across 10 years. Coral bleaching occurred at those sites over that time span (*48*), but symbiont genotypes in octocorals remained constant, which is consistent with our findings.

The ubiquity of *Breviolum* in Caribbean octocorals, the continued prevalence of *Breviolum* across a bleaching event, and the absence of symbiont shuffling during the bleaching event (in addition to the observed loss of *Cladocopium* among *P. dichotoma* colonies) point to a major role of *Breviolum* in determining holobiont susceptibility to thermal stress. *Breviolum* was traditionally characterized as a thermally sensitive genus (*49*), but those studies examined *Breviolum minutum*. There is considerable genetic diversity within the *Breviolum* B1 lineage (*22*–*24*) and other species of *Breviolum* are more thermally tolerant (*17*, *18*, *25*), with certain genotypes of *Breviolum antillogorgium* even exhibiting positive growth at 32°C (*25*–*27*). In our study, all three host species harbored symbiont species within the genus *Breviolum*, which may have contributed to the resilience of the symbiont populations and the overall resistance of the holobiont to thermal stress.

Cluster analysis showed that symbionts within the colonies of each species could be partitioned into groups on the basis of the genotypic composition, but this patterning did not contribute to the variance in cell densities. As noted in the results, cell densities were not significantly different between clusters within a host species, suggesting the slightly different combinations of symbiont genotypes were equally resilient to the stress. That is, while host species differed in their response to thermal stress, among individuals within a host species, specific hosts were not associated with differences in the bleaching or recovery based on the symbiont genotype that they harbored (Figs. 6 and 7 and fig. S7). However, it is important to note that the host also plays a role in holobiont responses to thermal stress (*17*, *18*, *23*, *33*, *47*, *50*, *51*). For instance, Kenkel *et al.* (*52*) found that inshore and offshore colonies of *Porites astreoides* were genetically differentiated and had symbionts that did not differ in *ITS* haplotype. Despite the similarity of the symbiont populations, there were differences in thermal tolerance between offshore and inshore colonies. While studies have found variation in thermal stress susceptibility not explained by symbiont genetic variation, note that many studies characterize symbiont diversity at either the genus or species level, identifying symbionts based on variation in symbiont nuclear 18*S rDNA*, *ITS2*, or cp-*23S rDNA*. Yet, substantial physiological variation exists even within a symbiont species (*25*–*27*, *35*, *36*), and the use of genetic markers with within-species genotypic resolution is necessary to fully understand variation in holobiont responses. Studies that have resolved the symbiont genotype at the within-species level indicate that the host-symbiont combination is important (*23*, *47*, *53*). For example, Parkinson *et al.* (*23*) found *Acropora palmata* colonies all harboring the same symbiont genotype varied in their response to cold shock, and Kavousi *et al.* (*47*), also using high-resolution markers to characterize symbiont variation, showed that response to thermal stress varies among different host-symbiont combinations. Future research should continue to explore the response to stress by examining within-species differences among host-symbiont pairs. In addition, a clearer understanding of how the holobiont responds to thermal stress can be gained by examining other physiological parameters [see, e.g., (*17*, *18*)] and changes in host and symbiont transcriptomics and expression of thermal genes (*54*, *55*). Last, other components of the holobiont also may contribute to the response to thermal stress. For example, studies have shown that thermally sensitive corals inoculated with the microbiome from thermal tolerant corals bleached less than control (thermally sensitive) individuals (*56*).

We differentiated the dynamics of host and symbiont in response to a thermal stress event. Our findings support the conclusion that, in general, Caribbean octocorals exhibit both resistance and resilience in the face of marine heat waves. This is consistent with previous studies that have shown a lack of bleaching among many octocoral species; octocoral bleaching, when it does occur, occurs later and after a longer exposure time than that of scleractinian corals, and in general, there is low mortality among octocorals that do bleach (*13*, *16*). Furthermore, we demonstrated that within octocorals, symbiont genotype does not change across a bleaching event. Our results emphasize that in understanding the response to marine heat waves, it is critical to follow individual colonies across an event with long-term monitoring of both host and symbiont response and examine this response at least at the level of symbiont species (if not genotype) to identify potentially resilient species. Work such as this will be critical to understanding the dynamics and response of corals to heat waves and making predictions about the impact of these events on coral reef community structure.

MATERIALS AND METHODS

Octocoral collections

In September 2014, extensive bleaching of reef cnidarians was observed in the Florida Keys (*57*, *58*). Notably, bleaching was observed in several species of octocorals that had rarely bleached in earlier warming events. To document the event, we tagged, recorded the bleaching status, and sampled 15 colonies at Alligator Reef in September 2014 and resampled these same colonies in February 2015 (table S1). In addition, in surveys conducted along established paths at five more sites in September 2014 (table S3), colonies were sampled, and bleaching status was recorded (table S2). A 3-cm piece of octocoral tissue was collected from below the tip of each colony and preserved in 95% ethanol for molecular analysis and host species identification following Bayer (*59*). Symbiont genera were determined in a subset of the samples collected in the 2014 site surveys and for all the tagged colonies at Alligator Reef in 2014 and 2015 (see below). Each colony was subjectively classified as bleached (BL) if greater than 75% of the colony appeared pale or

white, partially bleached (PB) if between 75 and 25% of the colony was pale or white, pale/necrotic (PN) if the colony was a pale color with dying tissue, and not bleached if less than 25% of the colony was pale (NB). On the basis of a return to normal coloration, most octocorals had recovered by May 2015. In anticipation of a second bleaching event predicted for fall 2015 (*60*, *61*), we expanded our monitoring of octocorals, examining three species on two patch reefs (both approximately 6 m in depth) in the middle Florida Keys. At site SC2 (N24 49.478, W 80.41.187), *P. dichotoma* (n = 14) and *M. elongata* (n = 13) colonies were marked with aluminum tags, while at site CMF (N24 44.701, W 80 46.806), *P. dichotoma* (n = 16) and *M. elongata* (n = 12) colonies were tagged (table S4). A third species, *M. atlantica* (n = 11 colonies), was added to the monitoring at CMF in September 2015, when severe bleaching was observed in this species (table S4). A 15-cm piece of each colony was initially collected to verify host species identification following Bayer (*59*). Except for *M. atlantica*, which was not sampled in May 2015, the tagged colonies were sampled before bleaching (May 2015), during bleaching (September 2015), and after bleaching (November 2015, March 2016, May 2016, September 2016, November 2016, March 2017, and August 2017). At each sampling point, 3-cm pieces of octocoral tissue were collected from a randomly selected branch of each colony. Samples were subdivided, with one piece preserved in 95% ethanol for molecular analysis and the other in 10% formalin for symbiont cell counts. Starting in September 2015, the coloration of each colony was compared to a CoralWatch coral health chart (*42*), and the matching color on the card was recorded.

Symbiont cell density

As bleaching is not always indicated by a change in color (*14*, *16*), bleaching was also characterized using symbiont density. The length and diameter of each sample were measured to the nearest 0.1 mm using vernier calipers, and the tissue was then homogenized in distilled water. The symbiont cells in the homogenate were counted at least four consecutive times, using standard hemocytometer procedures, and converted to cells per tissue volume using the formula for a cylinder to calculate tissue volume.

Temperature records

We deployed HOBO temperature loggers (Onset, Bourne MA) at each site in May 2015 and exchanged them at every collection period to obtain temperature recordings in situ. Temperatures at the two sites were examined visually, plotting the difference between the sites at each time point over the course of the study and then statistically using a generalized linear model (SPSS v21) comparing sites, months, and the interaction between the two. Differences between sites on specific months were based on 95% confidence intervals of estimated marginal means for each site/month comparison.

Using the temperature data, we calculated DHWs as a measure of accumulated heat stress (*62*). Following the procedure used by Gintert *et al.* (*57*) to determine DHW, we used data collected at the Molasses Reef C-Man station to compare the maximum running 30-day mean at our two study sites to that at Molasses Reef over the 2.25 years of our study. As suggested by Gintert *et al.* (*57*), we added the difference between our sites and the Molasses data (0.8° and 0.2°C for SC2 and CMF, respectively) to the maximum mean monthly (MMM) estimated for Molasses Reef (29.4°C) to calculate MMM for each site, resulting in a MMM of 30.2° and 29.6°C for SC2 and CMF, respectively. Following Skirving *et al.* (*63*), we used accumulation windows of 12 weeks in calculating DHW. Temperature data for SC2 between May 2016 and December 2016 are missing due to the loss of the logger.

Symbiont genera assignment and genotyping

We extracted symbiont DNA following Coffroth *et al.* (*64*). To determine symbiont genus, fragment size variation in the hypervariable region of domain V in the *chloroplast 23S rDNA* (cp-*23S rDNA*) was determined following Santos *et al.* (*65*). We used sequence variation in the domain V of the cp-*23S rDNA* and the B7Sym15 microsatellite flanking region to further characterize genotypic differences of symbionts within *Breviolum* B1. Samples were amplified in a 30-μl reaction volume. Amplification of the cp-*23S rDNA* followed Santos *et al.* (*66*), and amplification of the B7Sym15 microsatellite flanking region followed that of Pettay and LaJeunesse (*67*) except that the annealing temperature was 57°C. Products were Sanger-sequenced in both the forward and reverse direction (TACGen, Richmond CA). Sequences were assembled by trimming low-quality data and aligning forward and reverse reads for each sample to generate a consensus sequence, using Clustal Omega v. 1.2.2 (*68*) in Geneious Prime v. 2023.0.4 (*69*). Assembled sequences for both genes were deposited in GenBank (table S8). Then, all sequences for each locus were aligned using MAFFT v. 7 with the L-INS-I strategy (*70*, *71*). Alignments for both loci were concatenated and IQ-TREE v. 1.6.12 with ModelFinder with free rate heterogeneity enabled was used to identify substitution models for each locus and build a maximum likelihood tree with 1000 SH-aLRT branch test replicates and 1000 ultrafast bootstrap replicates. We used publicly available cp-*23S rDNA* and B7Sym15 flanking region sequences for *B. antillogorgium*, *Breviolum dendrogyrum*, *Breviolum endomadracis*, and *Breviolum meandrinium* as out-groups (table S8).

MLGs were constructed on the basis of allelic variation among microsatellite loci. *Breviolum*-specific microsatellite loci (B7Sym8, B7Sym15, B7Sym34, and Sym155) were used to generate MLGs for the symbionts in the *Muricea* spp. (*67*, *72*), and loci B7Sym9, B7Sym15, B7Sym36, and CA6.38 were used to construct MLGs for *P. dichotoma*'s symbionts (*67*, *72*, *73*). Amplification was performed in a volume of 10 μl using approximately 10 ng of DNA, 200 μM deoxynucleotide triphosphate, 1.5 mM (B7Sym34 and Sym155), 2 mM (CA6.38), 2.5 mM (all other loci) $MgCl_2$, 0.3 μM forward primer, 0.15 μM reverse primer, 0.15 μM fluorescent primer, *Taq* polymerase (0.5 U; New England Biolab), and 1× buffer (New England Biolab). Bovine serum albumin at 2.5% was also added to reactions using the B7Sym9 locus. Samples were denatured at 95°C for 2 min and then 30 cycles of 30-s denature at 95°C, 30-s annealing at 57°C for all but locus B7Sym9, where annealing was at 52°C, and 30-s extension at 72°C, with a final extension of 5 min at 72°C. All amplicons were visualized on a polyacrylamide gel, using the LI-COR NEN Global IR2 DNA Sequencer, and scored by eye alongside 50– to 350–base pair size standards (LI-COR Biotechnology) following the protocol of Santos and Coffroth (*73*). Each sample was assigned a specific MLG based on the allele(s) present at each locus. Members of *Breviolum* are haploid in the vegetative state (*73*), so we interpreted the presence of multiple alleles at a given locus as multiple symbiont genotypes within a host colony. For each colony, data were also coded for the presence/absence of

a given allele for each sample and used to generate a second set of MLGs representing allelic diversity.

Analysis of symbiont density

Symbiont cell densities of the colonies were compared over time, between host species and symbiont genotypes within species, relative to visible bleaching (i.e., bleaching card scores), and compared to temperatures expressed as DHWs. The relationship between cell density in the interval before mortality and survival was also assessed.

Cell densities over the course of the study were compared in a GENLMM for all three host species combined and then each host species separately using a repeated-measures linear mixed model with Tukey's post hoc comparisons to assess the effects of site, time, and their interactions (SPSS v28). The relationship between symbiont cell density and DHW was assessed using a generalized linear model with a gamma log-link distribution. Colonies were nested within sites with DHW as a covariate. In addition to the main effects, the interaction between DHW and the colony was also tested.

Octocorals are often pigmented, and symbiont loss (bleaching) does not necessarily lead to a change in color (*14*, *16*). Field assessments of bleaching can be subjective. To establish the usefulness of field assessments using the Coral Watch Bleaching Card, we used a general linear model in R v. 4.2.0 to compare bleaching card scores to the cell count density measurements.

Analysis of genotypic data

We assessed the relationship between symbiont genotype and cell densities before, during, and after bleaching by first examining which symbiont genotypes were present and then assessing whether they changed over the course of bleaching. For each time interval, colonies were coded for whether they contained a single genotype or multiple symbiont genotypes and whether the genotype(s) were present and changed between sampling intervals.

For each host species, we also coded each colony for the presence/absence of all known alleles before, during, and after the bleaching event (May 2015, September 2015, and November 2015, respectively), concatenating the data from each sampling time. This created a matrix with colonies as the rows and the columns representing the presence or absence of each allele, with the alleles repeated for presence/absence before, during, and after visible bleaching (May 2015, September 2015, and November 2015). Following the approach of Davies *et al.* (*74*), colonies were clustered on the basis of Jaccard dissimilarities among the allele presence-absence data with a hierarchical cluster analysis (Cluster in SPSS v28). We examined whether there were differences in cell density between the clusters with a general linear analysis of cell density on the three dates as a repeated measure for each colony. Cell densities were log-transformed for the analysis.

Symbiont allelic and genotypic diversity was regressed against cell density to test whether symbiont diversity was correlated with symbiont loss in *M. elongata* [as in (*75*)]. We conducted a linear mixed models analysis of symbiont density with colony and sample month as factors and the number of alleles as a covariate to determine whether symbiont density was correlated with symbiont allelic diversity. The analysis was then repeated using genotypic diversity as the covariate.

Mortality

Chi-square analyses were used to test whether mortality differed between host species, symbiont genotype, or symbiont allelic diversity (the number of symbiont alleles identified in each host). Although many MLGs were identified, most were restricted to a few colonies, and symbiont genotypes which were found in less than five colonies were combined into an "other" category of rare genotypes. In some cases, genotypes changed across sampling times. For colonies that died, we used the final recorded genotype before death, and for those colonies that survived, the genotype at the last sampling point was used.

To establish whether the loss of symbionts during the bleaching event was correlated with subsequent death, we compared symbiont density in September 2015 of those colonies that died over the course of the study with that of the surviving colonies. The comparison was made with both logistic regression and ANOVA (SPSS v28 for both tests). In addition, for colonies that died, we compared symbiont density in the month before death to symbiont density in all surviving colonies for the same month, analyzing the data for each month separately, as well as for all sample periods combined. Statistical analyses of mortality were restricted to *M. elongata* as the other species had too few instances of mortality (*M. atlantica*, 0; *P. dichotoma*, 3).

Supplementary Materials

This PDF file includes:

Figs. S1 to S7
Tables S1 to S8
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