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Community dynamics and physiology of *Symbiodinium* spp. before, during, and after a coral bleaching event

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Abstract

Community dynamics and physiology of *Symbiodinium* associated with *Orbicella* (= *Montastraea*) *faveolata* were examined before, during, and after a thermally induced coral bleaching event in Puerto Morelos, Mexico. We combined microsampling molecular genotyping with in situ pulse-amplitude modulated fluorometry to correlate colony variability of *Symbiodinium* population identities and the phenomena of partial coral bleaching. Pigmented nonbleached portions of *O.* (=*M.*) *faveolata* were compared with bleached portions of the same colony. During bleaching, maximum quantum yield of photosystem II (PSII; $F_v : F_m$) was significantly lower and highly variable (range 0.110 to 0.680) compared with previous summers in which coral bleaching was absent (range 0.516 to 0.661) and recovery (range 0.480 to 0.716). Differential susceptibility to environmental perturbation of $F_v : F_m$ corresponded to distinct genetic identities of *Symbiodinium*. Analysis of ribosomal deoxyribonucleic acid (rDNA) internal transcribed spacer 2 (ITS2) revealed regions of the coral colonies that had phylotype A3 prior to bleaching were more resistant to the bleaching perturbation than adjacent bleaching-prone patches that harbored phylotypes B17 and C7. During environmental perturbation, regions of the colonies containing predominantly *Symbiodinium* phylotypes A3 or D1a retained significantly higher $F_v : F_m$ values than adjacent regions with phylotypes B17 and C7. Following bleaching, rapid recovery of symbiotic algal densities greatly exceeded normal seasonal oscillations. During recovery we document shifts in *Symbiodinium* populations and increase prevalence of *Symbiodinium* types A3 and D1a, phylotypes known to have enhanced thermal tolerances. Thermal tolerance of *Symbiodinium* spp. influences the changes of coral–*Symbiodinium* communities during disturbance events and the dynamics of coral–*Symbiodinium* repopulation.

Increased sea temperatures in conjunction with high irradiance can disrupt the symbiosis of symbiotic dinoflagellates (genus: *Symbiodinium*) and their coral host. Coral–*Symbiodinium* physiology is altered during bleaching (loss of *Symbiodinium*, and/or their pigments), thereby decreasing the amount of photosynthetically derived carbon transferred to the coral host (reviewed by Brown 1997). Sustained stress causes a reduction in coral tissue growth, skeletal calcification, and fecundity while increasing coral susceptibility to disease and mortality (Szmant and Gassman 1990; Brandt and McManus 2009; Cantin et al. 2010). Corals are often able to recover from bleaching events, and associated genotypic shifts in *Symbiodinium* communities have been documented (Berkelmans and van Oppen 2006; LaJeunesse et al. 2009), as has the eventual return to the original *Symbiodinium* communities (Thornhill et al. 2006). Greater understanding *Symbiodinium* physiology, population dynamics, and bleaching recovery are of paramount importance for ecosystem-wide analyses of global climate change scenarios as predicted by the Intergovernmental Panel on Climate Change (IPCC 2007).

Experimental and field-based studies of coral bleaching events have highlighted physiological differences between genetically diverse *Symbiodinium* that associate with reef-building corals (Rowan 2004; Abrego et al. 2008; Howells et al. 2011). *Symbiodinium* is a highly diverse group of dinoflagellates consisting of nine major clades (A–I; Pochon and Gates 2010). The major *Symbiodinium* clades can further be divided using various rapidly evolving molecular markers into various phylotypes (LaJeunesse 2001; Sampayo et al. 2009; LaJeunesse and Thornhill 2011). Substantial research emphasis has focused on delineating thermally tolerant vs. sensitive coral–*Symbiodinium* associations (Berkelmans and van Oppen 2006; Abrego et al. 2008; Howells et al. 2011). *Symbiodinium* belonging to clade D has been of particular interest, and an increased prevalence of corals hosting symbionts within this clade has been documented in regions that regularly experience adverse environmental conditions (i.e., high temperatures and turbidity; Baker et al. 2004; Fabricius et al. 2004; LaJeunesse et al. 2010). Pacific corals with clade D *Symbiodinium* often have higher thermal tolerance than conspecific corals hosting other clades of *Symbiodinium* (Fabricius et al. 2004; Berkelmans and van Oppen 2006). Under nonstressful conditions, *Symbiodinium* clade D is often found to be in very low background densities (< 1% total *Symbiodinium* community) in many coral taxa (Mieog et al. 2007; LaJeunesse et al. 2009; Silverstein et al. 2012). Observations that clade D symbionts often become prevalent in corals following a bleaching event have prompted the

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hypothesis that corals may have the ability to acclimatize to warmer temperatures (Baker et al. 2004; Berkelmans and van Oppen 2006).

Most coral colonies have been found to associate with a single dominant *Symbiodinium* phylotype (but *see* Baker and Romanski 2007); however, *Orbicella* (= *Montastraea*, Budd et al. 2012) *faveolata* often simultaneously hosts three or more genotypically dominant *Symbiodinium* phylotypes (Rowan and Knowlton 1995; Toller et al. 2001*b*; Kemp et al. 2008). *Symbiodinium* associated with *O. faveolata* often have spatial diversification of symbiont phylotypes within coral colonies, influenced by localized irradiance patterns (Rowan and Knowlton 1995). Regions of *O. faveolata* colonies exposed to high irradiance often harbor phylotypes belonging to Clades A and B (Rowan and Knowlton 1995; Toller et al. 2001*b*; Kemp et al. 2008), while the lower irradiance sides of the same colonies often have phylotypes of Clade C. During coral bleaching events, localized or ''patchy'' bleaching of *O. faveolata* has been documented and correlates with distribution patterns of genetically distinct *Symbiodinium* phylotypes that exhibit differential thermal and irradiance tolerance (Rowan et al. 1997).

Following a coral bleaching event and the loss of millions of algal symbionts, vacated host tissue can become available for repopulation. Coral endodermal cells that have very low symbiont densities are presumably rich in holozoic derived inorganic metabolic waste that may facilitate the establishment and/or proliferation of thermally tolerant *Symbiodinium* (Piniak et al. 2003; Baker et al. 2013). Repopulation of *Symbiodinium* following bleaching events is not very well understood, and few studies have monitored population structure of *Symbiodinium* communities postbleaching. In the Caribbean, two multiyear ecological surveys of *Symbiodinium* community structure have documented the emergence of *Symbiodinium* internal transcribed spacer 2 (ITS2) type D1a as a dominant symbiont type in some corals, including *Orbicella* spp., with the onset of coral bleaching (Thornhill et al. 2006; LaJeunesse et al. 2009). In the 2–3 yr following bleaching events, the abundance of D1a in these same corals was found to decrease, as measured by denaturing gradient gel electrophoresis and semiquantitative polymerase chain reaction (PCR), and *Symbiodinium* populations appeared to eventually revert back to prebleaching genetic structure (Thornhill et al. 2006; LaJeunesse et al. 2009). These results suggest that postbleaching shift(s) in dominant *Symbiodinium* populations associating with *Orbicella* spp. tend to be temporary and will shift back to prebleaching dominance if bleaching perturbation does not occur in subsequent years.

Here, we report the effects of coral bleaching on the diverse *Symbiodinium* community of *O. faveolata* in Puerto Morelos, Mexico. This work substantially extends the utility of microsampling techniques and provides a novel approach that simultaneously documents shifts in intracolonial *Symbiodinium* communities, bleaching-sensitive localized regions within colonies, and repopulation of thermally tolerant *Symbiodinium* phylotypes following a coral bleaching event. Furthermore, to investigate functional differences and recovery patterns of genetically diverse *Symbiodinium* associating with *O. faveolata*, we examined intracolonial variability in *Symbiodinium* cell density, chlorophyll *a* (Chl *a*), and maximum quantum yield of photosystem II (PSII; $F_v : F_m$) throughout the coral colony before, during, and after a severe coral bleaching event that occurred during the summer of 2009.

Methods

Symbiodinium *quantification and Chl* a *determination*—Six large (2–4 m) hemispherical colonies of the dominant reef-building coral *O. faveolata* were randomly selected and tagged for repeated sampling from La Bocana Reef, Puerto Morelos, Mexico (2–4 m depth; 20.5228°N, 86.5105°W). These colonies were sampled seasonally (three to four times a year) from 2007 to 2010. Samples were removed from the uppermost portions of the colony using a hammer and chisel (approximately 5–8 cm in diameter). During a coral bleaching event (October 2009) two fragments per colony were chiseled from tagged colonies. To compare bleached and nonbleached regions of *O. faveolata* colonies, one sample was taken from a pigmented region of the coral and a second sample was taken from a visually bleached region of the same colony. Coral tissue was removed using a Waterpik™ (Waterpik) and filtered seawater (0.45 μm; Johannes and Wiebe 1970). The resulting slurry, containing coral tissue and symbiotic algae, was homogenized for approximately 10 s using a Tissue Tearror (BioSpec Products™). Aliquots (1 mL) were taken from homogenized slurry and preserved with formalin. *Symbiodinium* density of preserved samples was quantified microscopically via eight replicate hemocytometer counts (AO Spencer Bright Line Improved Neubauer hemocytometer) and normalized to coral surface area by correlations between weight and surface area of aluminum foil imprints (Marsh 1970).

To compare Chl *a* content of bleached and nonbleached coral samples, two replicate 15 mL subsamples of the coral slurry were centrifuged (1500 rotations per minute (rpm) for 5 min) to pellet *Symbiodinium*, the supernatant discarded and the pellet frozen until further analysis. Chlorophyll was extracted using 100% acetone and mild sonication for approximately 10 s using a Sonicator Model W-225 (Heat Systems–Ultrasonics) at 20% (frequency 20 KHz; power 200 Watts), placed in the dark at −20°C for 24 h, and then centrifuged to remove particulate matter (1500 rpm for 5 min). Sample supernatants were measured for chlorophyll absorbance with a BioRad SmartSpec™ 3000 spectrophotometer (BioRad) at 630, 663, and 750 nm, and Chl *a* content was quantitated using the equations of Jeffrey and Humphrey (1975) for dinoflagellates, then normalized to coral surface area as described above (Marsh 1970).

Microsampling procedure and in situ pulse-amplitude modulated (PAM) measurements—The localized distribution of *Symbiodinium* phylotypes within a single coral colony was examined using the microsampling procedures of Kemp et al. (2008). Tissue from two to three polyps of *O. faveolata* was collected from four of the six colonies that were sampled with a hammer and chisel for seasonal

Symbiodinium densities and Chl *a* concentration. The four colonies selected were not shaded from neighboring colonies and were hemispherical with minimal undulations. Coral polyp removal was performed every 20 cm using 2 cc syringes with 16 gauge needles along fixed axes from top to bottom of each colony at north, east, south, and west compass bearings. Owing to the large size of the colonies, there was no observable overlap or interference between coral samples taken using a hammer and chisel and microsamples (two to three polyps) taken along axes. Corals were sampled in September 2007 (nonbleached), October 2009 (bleached), and December 2009 (recovery). The maximum quantum yield of PSII ($F_v : F_m$; $F_v = F_m - F_0$) was measured directly adjacent to microsamples using saturation pulses from a PAM fluorometer (saturation width 0.85 s of > 4500 μmol photons m^{-2} s^{-1} saturation light pulse; Diving PAM; Walz). To ensure that nonphotosynthetic quenching was suppressed and that corals were adequately dark adapted, all measurements were conducted at least 15 min after sunset. All in situ measurements were made under light conditions < 5 μmol photons m^{-2} s^{-1} as measured with diving-PAM fiber quantum photosynthetic active radiation sensor calibrated against a Li-Cor cosine-corrected light sensor.

When syringe samples were returned to the laboratory, they were transferred into 2 mL tubes and centrifuged at ~ 5000 *g* for 2 min. Supernatants were decanted, and algal pellets were preserved in dimethyl sulfoxide (DMSO) buffer (20% DMSO, 0.25 mol L^{-1} Ethylenediaminetetraacetic acid in saturated aqueous sodium chloride).

Genetic identification of Symbiodinium—Dominant phylotypes of *Symbiodinium* in each tissue sample were identified as described by LaJeunesse et al. (2003). Deoxyribonucleic acid (DNA) was isolated using a modified Promega Wizard genomic DNA extraction protocol (Promega Corporation) and the PCR amplified ITS2 region of nuclear ribonucleic acid (RNA) genes was analyzed via denaturing gradient gel electrophoresis (DGGE; LaJeunesse et al. 2003). PCR amplification of the ITS2 region was accomplished using a touchdown thermal cycle described in LaJeunesse et al. (2003) with the forward primer "ITSintfor2" (5′-GAATTGCAGA ACTCCGTG-3′) and the reverse primer "ITS2CLAMP" (5′-*CGCCCGCCGC GCCCC-GCGCC CGTCCCGCCG CCCCCGCCC* GGGATCCATA TGCTTAAGTT CAGCGGGT-3′), with a 39 base pair guanine and cytosine (GC) clamp (italicized) (LaJeunesse et al. 2003). Using a CBS Scientific™ (CBS Scientific Company) system, products were electrophoresed on 45–80% urea-formamide gradient denaturing gradient gels (100% consists of 7 mol L^{-1} urea and 40% deionized formamide) for 10 h at 150 V at a constant temperature of 60°C (LaJeunesse et al. 2003). Samples were run parallel to standards from identical corals, whose prominent DGGE bands had been previously sequenced.

Statistical analysis—All statistical analyses were performed using Sigma-Stat (version 3.1; Systat Software). All data sets satisfied assumptions of normality and equal variance, except where noted. Seasonal symbiotic algae densities were found not to have equal variance and therefore were compared using Friedman repeated measures analysis of variance (ANOVA) on ranks. Bleached vs. nonbleached paired coral samples were analyzed using a paired *t*-test. $F_v : F_m$ from nonbleached (September 2007), bleached (October 2009), and recovery periods (December 2009) were found to not be normally distributed and failed equal variance. Consequently, nonparametric comparisons using Kruskal–Wallis one-way ANOVA on ranks was used. The correlation between *Symbiodinium* identity and within-colony comparisons of $F_v : F_m$ along directional axes was analyzed using one-way ANOVA or Kruskal–Wallis one-way ANOVA on ranks when data were not normally distributed and/or failed equal variance. Tukey or Dunn's method post hoc multiple comparisons were performed whenever overall treatment effects were found. To test for *Symbiodinium* community deviation during bleaching and recovery, a Pearson's chi-squared (χ^2) analysis was used. September 2007 (nonbleached) dominant *Symbiodinium* phylotype assemblages ($n = 4$; 74 individual samples) were used to formulate a null hypothesis of *Symbiodinium* communities within *O. faveolata,* and χ^2 was used to test for deviation from this assemblage. A significance level α, no greater than 0.05, was used for all statistical treatments.

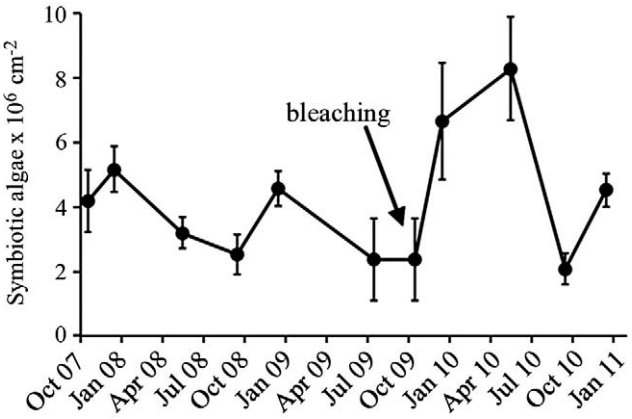


Fig. 1. Seasonal *Symbiodinium* cell densities for *O. faveolata* sampled from uppermost nonbleached portions of the colonies. Cell density data expressed as means ± 95% confidence intervals ($n = 6$).

Results

Symbiodinium *densities and Chl* a *content—Symbiodinium* densities fluctuated seasonally and were normally the highest in winter ($5.2 \times 10^6 \pm 1.0$ standard deviation (SD) cells cm^{-2}, December–February) and lowest in the summer months ($2.7 \times 10^6 \pm 0.85$ SD cells cm^{-2}, July–October; Fig. 1). Postbleaching comparisons of bleaching and recovery showed a threefold increase in *Symbiodinium* densities from October 2009 to December 2009 (Tukey post hoc multiple comparison, $p < 0.05$). Postbleaching *Symbiodinium* cell densities of May 2010 ($8.3 \times 10^6 \pm 2.0$ SD cells cm^{-2}) were compared with prebleaching May 2008 ($3.2 \times 10^6 \pm 0.62$ SD cells cm^{-2}) densities and found to be significantly higher than expected seasonal oscillation, indicating continuation of postbleaching cell repopulation and potential disequilibrium (Tukey post hoc multiple

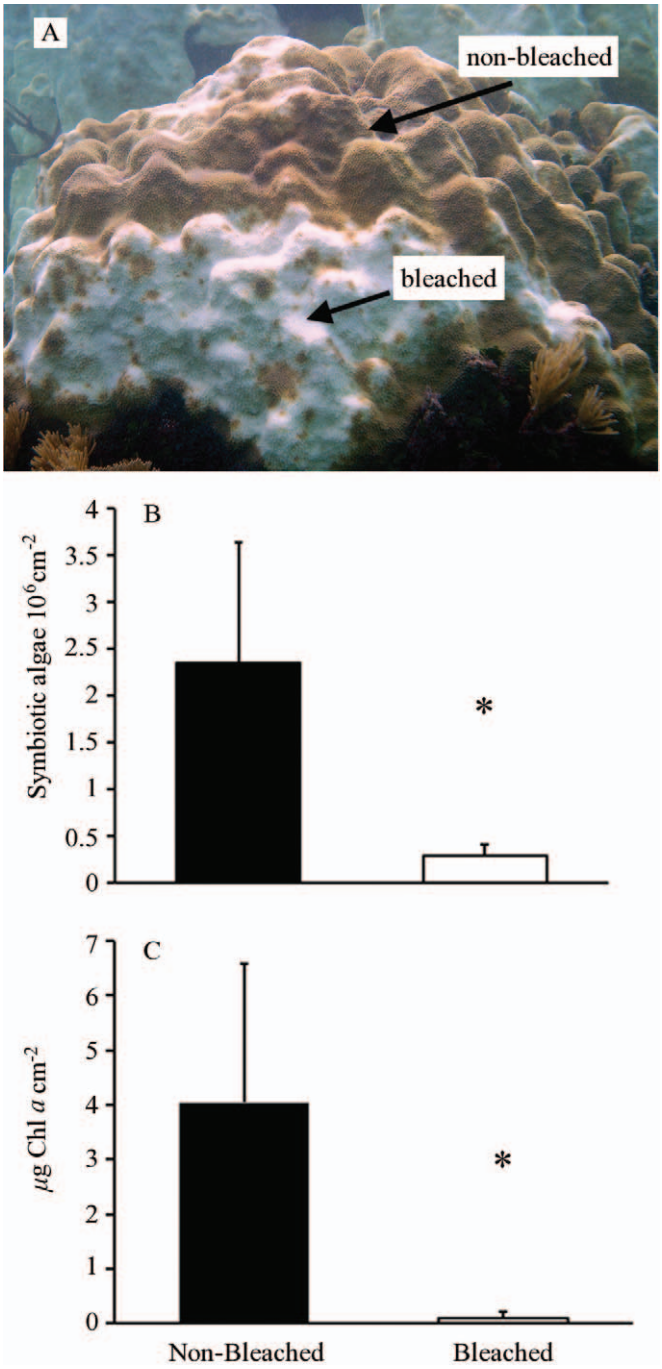


Fig. 2. (A) Bleached and nonbleached regions of a colony of *O. faveolata* sampled for analysis. One sample of each region was taken per coral ($n = 6$). (B) *Symbiodinium* cell density from these regions. Cell density data expressed as means ± 95% confidence intervals ($n = 6$; paired *t*-test, $t = 3.326$, df = 5, $p < 0.05$). (C) Chl *a* content of corals per unit area from nonbleached and bleached regions of sampled corals ($n = 6$; paired *t*-test, $t = 2.975$, df = 5, $p < 0.05$). An asterisk indicates a statistically significant difference between nonbleached and bleached regions of coral colony ($p < 0.05$).

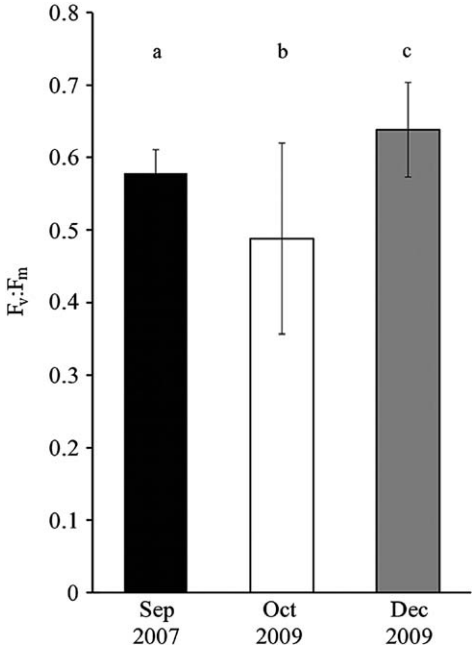


Fig. 3. Maximum quantum yield of PSII ($F_v : F_m$), measured along fixed axes from top to bottom of the colony. September 2007 ($n = 4$; 83 samples), October ($n = 4$; 83 samples), December ($n = 4$; 63 samples) are individual bars, and $F_v : F_m$ are expressed as means ± SD. Dissimilar letters at the top of each histogram denote statistical differences (Dunn's post hoc comparison; $p < 0.05$).

comparison, $p < 0.05$; Fig. 1). Intracolonial comparisons of bleached corals (October 2009) revealed *Symbiodinium* densities were eight times higher in nonbleached vs. bleached areas of the colonies (paired *t*-test, $t = 3.326$, degrees of freedom (df) = 5, $p < 0.05$). Similarly, Chl *a* (μg cm^{-2}) was found to be 44 times higher in nonbleached regions compared with bleached regions (paired *t*-test, $t = 2.975$, df = 5, $p < 0.05$; Fig. 2).

Coral–Symbiodinium *PAM fluorescence measurements*—The mean maximum quantum yield of PSII ($F_v : F_m$), measured along fixed axes from top to bottom of the colony, showed less intracolonial and intercolonial variability during both prebleaching (September 2007) and recovery (December 2009) sampling periods (Figs. 3–6). Mean $F_v : F_m$ values were 0.58 ± 0.03 SD in September and 0.63 ± 0.08 SD in December. This is in contrast to high, intracolony $F_v : F_m$ variability documented during the bleaching event of October 2009, when mean values were 0.49 ± 0.13 SD, but intensely bleached areas exhibited $F_v : F_m$ levels as low as 0.11, while unbleached areas were at or above 0.50. Comparisons between each time point revealed significant overall differences in $F_v : F_m$ (Dunn's post hoc comparison; $p < 0.05$; Fig. 3). The amount of $F_v : F_m$ intracolonial variability documented in bleached corals was 4.3 times greater than the prebleaching summer of 2007 and 1.7 times greater than postbleaching recovery (December 2009). Mean within-colony $F_v : F_m$ values were measured along fixed directional axes and were found to not be statistically different for all axes and time points

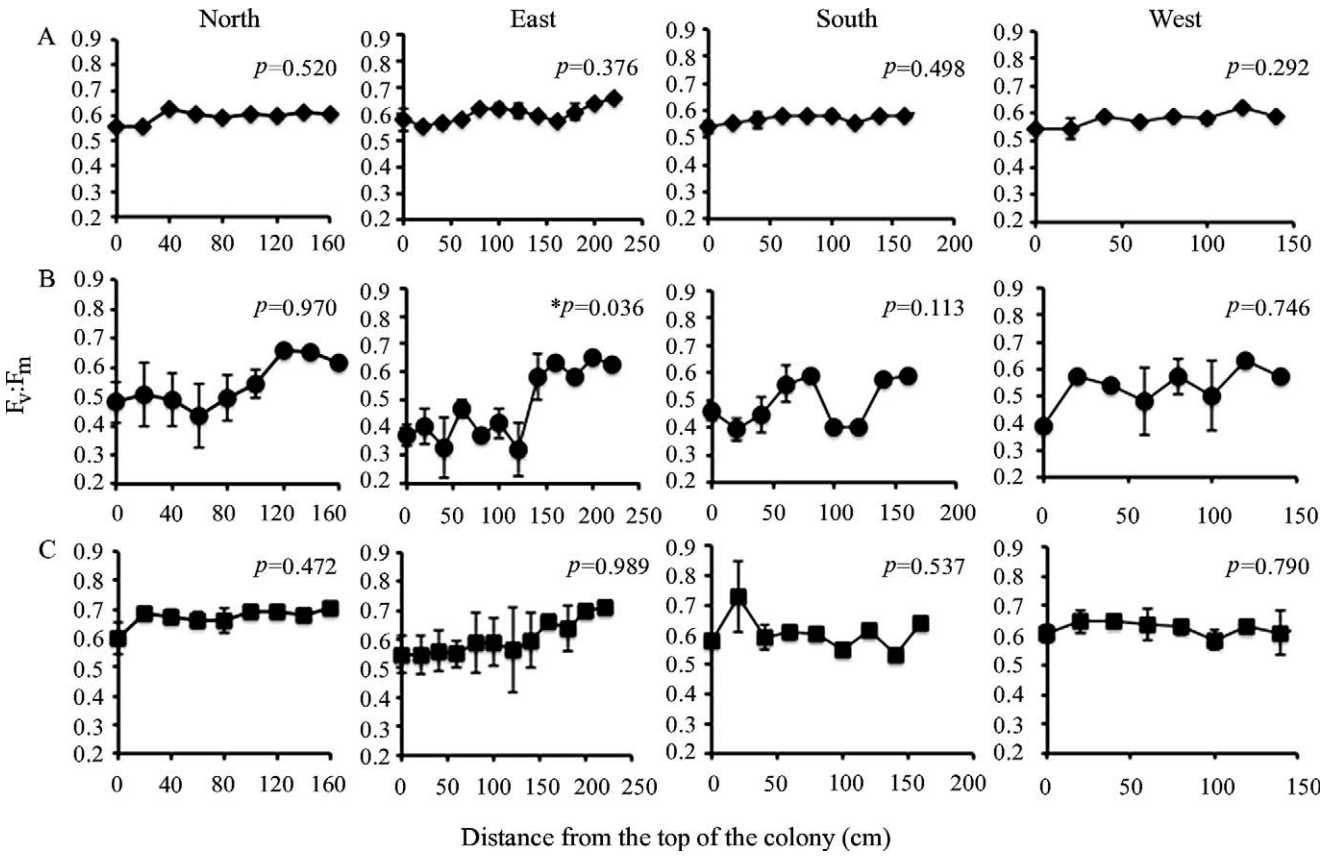


Fig. 4. Within-colony comparisons of maximum quantum yield of PSII ($F_v : F_m$), measured along fixed axes from top to bottom of colony along north, east, south, west axes. (A) September 2007 ($n = 3$), (B) October 2009 ($n = 3$), (C) December 2009 ($n = 3$). Individual points are means ± standard error. Owing to coral colony heterogeneous morphology individual points have 1–3 $F_v : F_m$ measurements. One-way ANOVA or Kruskal–Wallis one-way ANOVA on ranks were used to compare within-colony differences in $F_v : F_m$. An asterisk indicates a statistically significant difference (one-way ANOVA; $p < 0.05$).

except for east axes measured during bleaching (October 2009; One-way ANOVA; $p < 0.05$; Fig. 4). No significant difference in $F_v : F_m$ was found between regions hosting different phylotypes of *Symbiodinium* during September 2007 (Figs. 5A, 6A). However, during bleaching (October 2009) regions of the coral that had phylotypes A3 or D1a (sensu LaJeunesse 2001) showed significantly higher $F_v : F_m$ values than regions containing B17 or C7 (Dunn's post hoc comparison; $p < 0.05$; Figs. 5B, 6B). During recovery (December 2009) regions that contained phylotypes A3 or D1a had significantly greater $F_v : F_m$ values than regions containing solely B17 (Dunn's post hoc comparison; $p < 0.05$; Figs. 5C, 6C). This variability in $F_v : F_m$ indicates differential susceptibility among coral and associated *Symbiodinium* phylotype(s) to the environmental perturbation (Figs 5, 6).

Prebleached, bleached, and recovery of Symbiodinium *communities*—Differences in dominant *Symbiodinium* populations associated with *O. faveolata* were observed between prebleached, bleached, and recovery events (χ^2; df = 5, $p < 0.001$; Fig. 7). Prevalent shifts in *Symbiodinium* populations were observed between all phylotypes. In September 2007 during nonbleaching conditions phylotype A3 was detected in 15% of all microsamples, B17 was found in 73%, C7 in 38%, and D1a was not detected in any of the samples using DGGE analysis (*see* Thornhill et al. 2006; LaJeunesse et al. 2009 for detection thresholds). During the bleaching event of October 2009, *Symbiodinium* type A3 was found in 43% of the microsamples, B17 was found in 48%, C7 in 4%, and D1a in 9%. During recovery in December 2009 *Symbiodinium* type A3 was detected in 32% of the microsamples, B17 was found in 47%, C7 in 7%, and D1a in 21% (Fig. 7).

Discussion

The functional and genetic diversity of coral–*Symbiodinium* symbiotic associations is an area of intense research. Often, *O. faveolata* colonies simultaneously host up to three dominant phylotypes of *Symbiodinium* (Rowan and Knowlton 1995; Toller et al. 2001*b*; Kemp et al. 2008). This exceptional ability to host diverse dominant populations, at over 5% of the total *Symbiodinium*, makes it an ideal coral to study differential susceptibility of *Symbiodinium* to environmental perturbations. We document how stable, genetically diverse *Symbiodinium* communities associating with *O. faveolata* are significantly altered during and following a coral bleaching event. Herein, measurements of *Symbiodinium* cell densities prior to bleaching (October 2009) showed seasonal oscillations similar to those described by Fitt et al. (2000). Two months

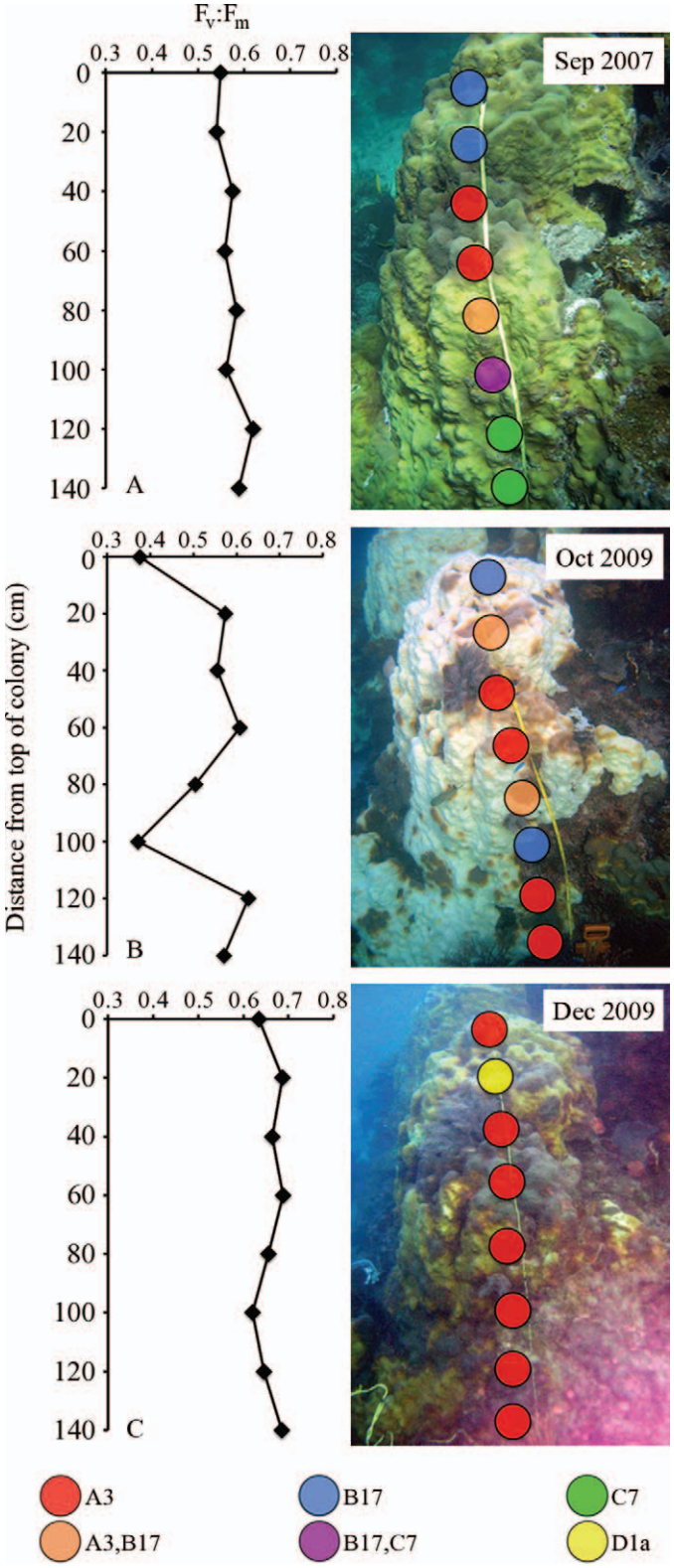
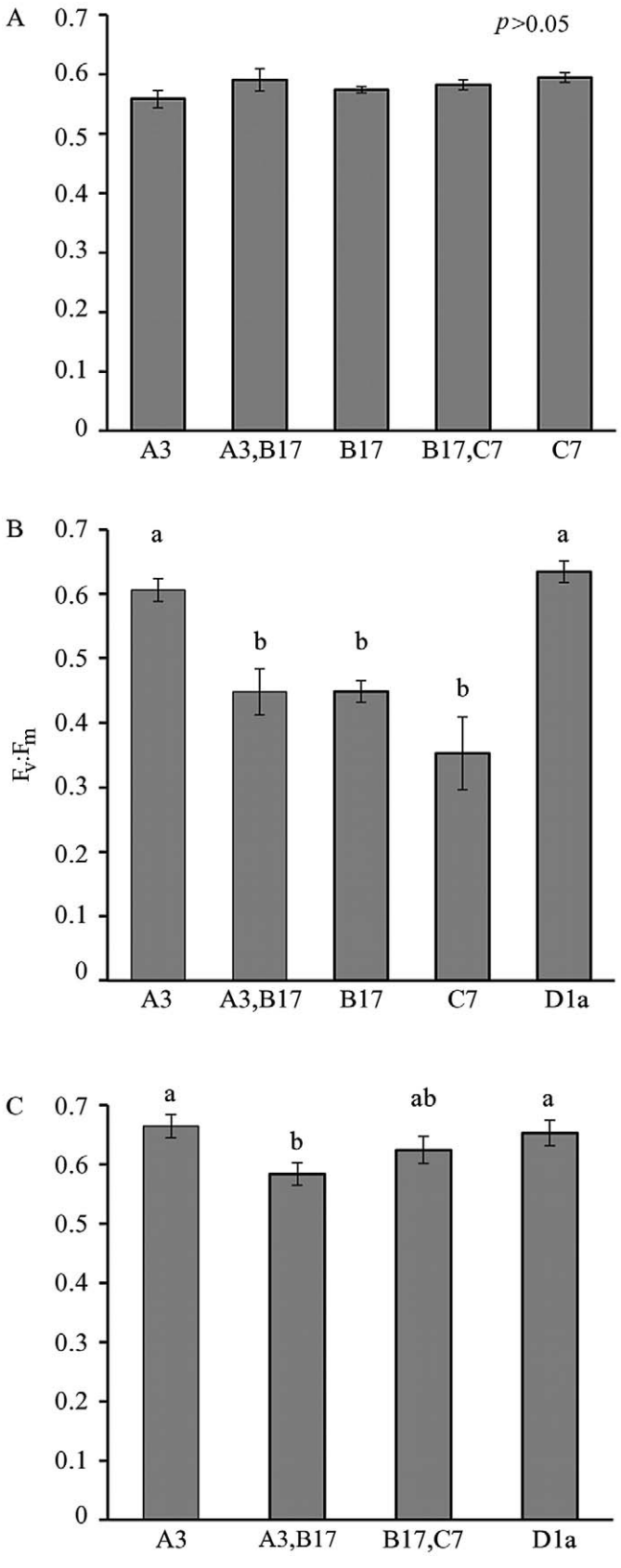


Fig. 5. Sampling axes along *O. faveolata* colonies (A) September 2007, (B) October 2009, and (C) December 2009. Microsamples were collected every 20 cm for *Symbiodinium* identification, represented by colored symbols, and in situ fluorescence measurements were taken directly adjacent to microsamples. Vertical line graphs are $F_v:F_m$ measurements aligned with *Symbiodinium* phylotypes within a single coral colony (from top to bottom) to demonstrate within-colony variability that correlated with *Symbiodinium* type.

after bleaching, when temperatures were no longer stressful (December 2009), symbiont density increased 28–48%, a greater augmentation than during any other December for which we sampled (2007–2008 and 2010). Moreover, in May 2010 (7 months after bleaching) samples were found to have a 159% increase over that of May 2008 (Fig. 1). This rapid ability to repopulate the vacated tissue demonstrates the rapid recovery capabilities that coral–*Symbiodinium* populations retain and may be critical in determining survivorship of the coral colony (Fitt et al. 1993; Rodriguez-Roman et al. 2006).

Susceptibility to bleaching showed large amounts of within-colony variability during the 2009 bleaching event. Areal cell densities and Chl *a* from pigmented, nonbleached regions of coral colonies were found to range from 1.1 to 5.0 $\times$ 10^6 cm^{-2} and 0.8 to 10.0 μg Chl *a* cm^{-2}, respectively (Fig. 2). Such large variation within what is commonly distinguished as nonbleached (i.e., pigmented) calls attention to the complex nature of coral–*Symbiodinium* physiology during an environmental perturbation resulting in bleaching. Nonbleached regions of the coral had eight times the areal density of *Symbiodinium* and 22 times higher Chl *a* content than bleached regions of the same coral (Fig. 2). During bleaching we also documented within-colony variability in maximum quantum yield of PSII ($F_v:F_m$) (Figs. 4, 5). Regions of coral colonies that had phylotypes A3 or D1a retained significantly higher $F_v:F_m$ than regions containing high abundances of phylotypes B17 or C7 (Figs. 5, 6). Two months following bleaching we documented a community-wide increase in prevalence of the A3 and D1a *Symbiodinium* phylotypes (Figs. 5, 6). Together, these data suggest thermally tolerant *Symbiodinium* may be capable of rapid proliferation that allows the relative abundances of these phylotypes to increase. Furthermore, the repopulation of residual algal populations to adjacent tissues is apparent, possibly facilitated by animal metabolites derived from holozoic feeding (Piniak et al. 2003; Baker et al. 2013).

Current classification of *Symbiodinium* divides the group into nine clades (A–I; Pochon and Gates 2010). Of these, many phylotypes of A and D exhibit enhanced thermal tolerance in corals (Rowan et al. 1997; Berkelmans and van Oppen 2006; Reynolds et al. 2008). By using DGGE methodology, our study is unable to give absolute abundances of individual *Symbiodinium* phylotypes. To date, DGGE detection thresholds using ITS2 region for phylotypes belonging to Clades B, C, and D have been measured. Thornhill et al. (2006) and LaJeunesse et al. (2009) calculated that the detection thresholds are 6.3% for B1, 3.8% for C2, and 20% for D1a of the total *Symbiodinium* populations. It is likely that additional *Symbiodinium* phylotypes coexist at low abundances and go undetected using DGGE methodology (Mieog et al. 2007; Silverstein et al. 2012). Although the sensitivity of detection differs between *Symbiodinium* phylotypes, using



DGGE methodology is highly sufficient for detecting dominant phylotypes of *Symbiodinium* (*see* Sampayo et al. 2009; LaJeunesse and Thornhill 2011) and detecting large-scale population shifts in *O. faveolata–Symbiodinium* communities (Thornhill et al. 2006; LaJeunesse et al. 2009).

During the nonbleaching summer of September 2007, phylotype D1a was not found in any of our samples, but it may have been present in low-density background abundances undetectable by DGGE (Mieog et al. 2007; LaJeunesse et al. 2009; Silverstein et al. 2012). In comparison, we did detect phylotype D1a in 21% of postbleached samples. Similarly, we found dramatic increases of detectable *Symbiodinium* type A3 populations during bleaching–recovery processes. Phylotype A3 was identified in 15% of prebleaching samples, 42% of bleaching samples, and 32% of postbleaching symbiotic algae samples (Fig. 7). Conversely, phylotype B17 dropped from being detected in greater than 70% of the samples to less than 50% of the samples upon bleaching and recovery. Phylotype C7 decreased the most, was detected in $< 5\%$ of bleaching samples, and did not show signs of rapid recovery prevalence (Fig. 7). Although we are unable to determine absolute proportion of individual *Symbiodinium* phylotypes throughout the environmental perturbation and recovery, it was evident that community-wide shifts in dominant *Symbiodinium* populations occurred. These findings corroborate with functional diversity and recovery patterns previously documented by Rowan et al. (1997), Toller et al. (2001*a*), and LaJeunesse et al. (2009).

Symbiodinium belonging to clade A is commonly associated with shallow-water corals in the Caribbean, leading to the hypothesis that many *Symbiodinium* belonging to clade A are high-irradiance and high-temperature specialists (LaJeunesse 2002). In support of this hypothesis, physiological studies of cultured isolates and in hospite *Symbiodinium* have shown that many phylotypes of clade A *Symbiodinium* are high-light and high-temperature tolerant and facilitated by enhanced photoacclimation and photoprotective pathways (Robison and Warner 2006; Reynolds et al. 2008; Takahashi et al. 2009). Our field measurements of $F_v : F_m$ support experimental work on clade A phylotypes. During the environmental perturbation that triggered bleaching in October 2009 regions of *O. faveolata* colonies that had phylotype A3 had significantly higher $F_v : F_m$ values than regions containing phylotypes B17 and/or C7 (Figs. 5, 6). During bleaching the greater thermal tolerance of phylotype A3 documented by $F_v : F_m$ likely explains much of the localized regions of coral colonies that retained pigmentation and appeared "patchy" in nature (Fig. 5). Therefore, the postbleaching increase in A3 detected in this

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Fig. 6. Maximum quantum efficiency of photosystem II ($F_v : F_m$) measurements from different *Symbiodinium* phylotypes sampled in situ along directional fixed axes of *O. faveolata*. Data are means ± standard error (SE) (A) September 2007 ($n = 3$; 68 samples), (B) October 2009 ($n = 3$; 64 samples), (C) December 2009 ($n = 3$; 48 samples). Dissimilar lowercase letters denote statistical differences between *Symbiodinium* phylotypes (Dunn's post hoc comparison; $p < 0.05$).

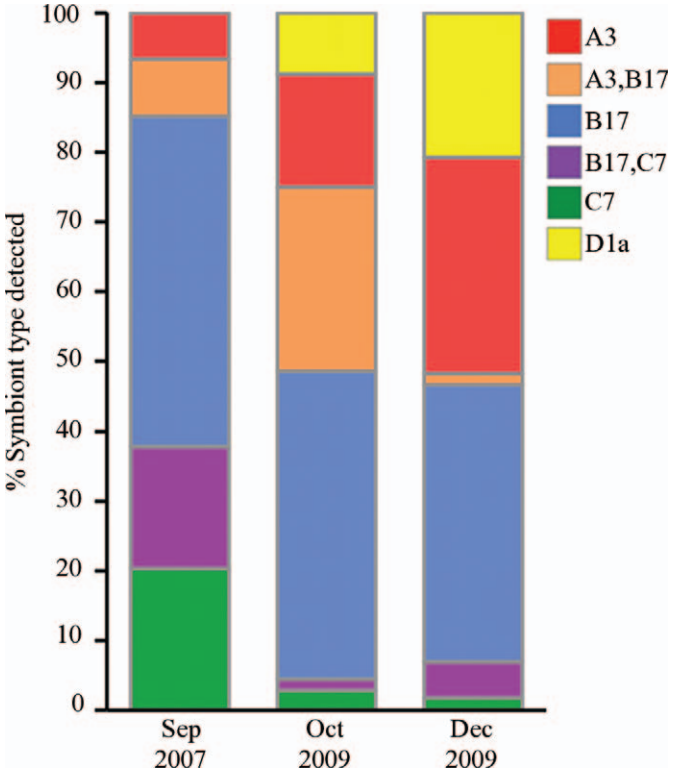

Fig. 7. Compilation of *Symbiodinium* (ITS2 phylotypes) communities from *O. faveolata* (La Bocana Reef, Puerto Morelos, Mexico) sampled along fixed axes of individual colonies. Samples were taken every 20 cm from top to bottom along north, east, south, west axes of each colony ($n = 4$) using microsampling techniques. Identical coral colonies from along fixed axes were sampled in September 2007 (prebleached; 74 samples), October 2009 (bleached; 68 samples), and December 2009 (recovery; 58 samples). Bar graphs represent total percent abundance of *Symbiodinium* phylotypes detected using DGGE from all coral colonies sampled regardless of position. Dissimilar lowercase letters denote statistical differences in overall dominant *Symbiodinium* phylotypes between sampling periods (χ^2; df = 5, $p < 0.001$).

study is likely due to the enhanced photoprotection and cyclic electron transport capabilities of this phylotype.

During bleaching conditions, enhanced photoprotective traits of *Symbiodinium* phylotypes A3 and D1a likely facilitate the proliferation and recruitment to vacated host tissue previously occupied by thermally sensitive phylotypes B17 and C7. Differential growth rates between symbiont phylotypes are likely exacerbated by differential propensity vs. resistance to photoinhibition and could account for apparent competitive exclusion or inclusion within a single coral colony. Accordingly, environmental fluctuations and microhabitat heterogeneity should lead to symbiotic algal nonequilibrium within individual colonies that may facilitate coexistence of multiple phylotypes of endosymbiotic dinoflagellates associated with *O. faveolata.* This conjecture is similar to the "paradox of the plankton" that Hutchinson first identified, which led him to ask why so many species coexist in a supposedly homogenous habitat (Hutchinson 1961).

The environmentally dynamic within-colony heterogeneity of *Symbiodinium* associated with the *Orbicella annularis* "complex" (including *O. faveolata*) has been shown to be more flexible than most corals and is frequently found to associate with one to four dominant phylotypes of *Symbiodinium* (Rowan and Knowlton 1995; Toller et al. 2001*b*; Kemp et al. 2008). Owing to the large boulder nature of *O. faveolata* colonies, large gradients of incident irradiance exist that result in corresponding *Symbiodinium* spp. zonation patterns and photoacclimation traits (Rowan and Knowlton 1995). It is important to recognize that although species of the *O. annularis* "complex" regularly host multiple, dominant *Symbiodinium* phylotypes, many of these *Symbiodinium* have been found to be host specific, only associating with *Orbicella* spp. (e.g., B17 and C7), suggesting host–symbiont coevolution (Thornhill et al. 2014).

Differential reduction in maximum quantum yield of PSII ($F_v : F_m$) indicates probable photoinhibition and photodamage incurred by thermally susceptible *Symbiodinium* (Figs. 5, 6) (Iglesias-Prieto et al. 1992; Warner et al. 1999; Smith et al. 2005). These observations highlight the importance of physiological differences within genetically diverse types of *Symbiodinium.* Under nonstressful conditions, thermally sensitive *Symbiodinium* phylotypes B17 and C7 are dominant. It is only during and after a bleaching event that phylotypes D1a and A3 proliferated to greater percent abundance and repopulated the adjacent host tissues vacated by phylotypes B17 and C7 *Symbiodinium* (Figs. 5, 7). Our findings corroborate with the findings of Rodriguez-Roman et al. (2006) that showed similar *Symbiodinium* population shifts following experimental bleaching. Our data demonstrate, in situ, how thermal tolerance may influence *Symbiodinium* populations. Furthermore, enhanced photoprotection and cellular repair mechanisms likely give a proliferation advantage in coral tissue repopulation following environmental perturbation. Rapid repopulation of *Symbiodinium* following bleaching is important not only for nutritional inputs to corals, but also for the absorption of potentially harmful irradiance (exacerbated by the loss of algae) that may cause host damage and a reduction of photosynthetic function (Rodriguez-Roman et al. 2006).

Our findings are similar to those of Thornhill et al. (2006) that documented shifts in *Symbiodinium* populations in *Orbicella* spp. after a bleaching event. Interestingly, Thornhill et al. (2006) showed shifts in *Symbiodinium* populations following bleaching were temporary in nature. Without subsequent bleaching episodes, all coral colonies in that study reverted back to the original dominant *Symbiodinium* phylotypes 2–4 yr after the environmental perturbation. Therefore, a reasonable conjecture would be that we would see similar results on the corals we studied, and without additional bleaching episodes *Symbiodinium* communities would revert to those documented in September 2007. Alarmingly, the frequency of coral bleaching events is predicted to increase over the next several decades (Hoegh-Guldberg et al. 2007). The increase of these environmental perturbations would potentially have important consequences on the *Symbiodinium* community structure of *O. faveolata*. The potential physiological and ecological trade-offs that may alter dominant *Symbiodinium* communities is not completely understood. Studies of juvenile *Acropora millepora* that had thermally tolerant

strains of *Symbiodinium* acquired less inorganic carbon and grew at slower rates than juvenile corals with more thermally sensitive strains (Little et al. 2004; Cantin et al. 2009). If adult corals have similar results by hosting thermally tolerant endosymbionts (e.g., D1a), then shuffling *Symbiodinium* community structure following a bleaching event may change how we currently perceive ''recovered'' coral (i.e., regained pigmentation) and may not accurately reflect a coral's physiological state.

When corals are bleached, they retain the ability to derive nutrients heterotrophically. The limiting factor(s) for persistence of residual *Symbiodinium* are cellular division rates (algal and host controlled), available space in the host, and the cell size (Falkowski et al. 1993; Smith and Muscatine 1999). During recovery (December 2009) we documented a threefold increase in *Symbiodinium* density that exceeded typical December seasonal oscillation from the identical coral colonies. This overcompensation during repopulation may indicate disequilibrium in host–symbiont regulation and warrants further genotype specific investigation of algae nutrient use and cellular division. Since symbiotic algal densities are drastically reduced during bleaching, availability of intracellular inorganic nutrients derived from holozoic feeding may be enhanced (Grottoli et al. 2006; Houlbreque and Ferrier-Pagès 2009). These findings corroborate with Fitt et al. (1993), who found mitotic indices of symbiotic algae from reef-building corals in the Florida Keys were inversely proportional to algal densities following bleaching, suggesting algae at high densities are more nutrient limited.

Community shifts of within-host *Symbiodinium* populations during bleaching and recovery likely reflects the trade-off cost of enhanced thermal tolerance, photoprotective traits, superior cellular repair mechanisms, host selectivity, and that of symbiont proliferation rates. *Symbiodinium* phylotypes, like other phytoplankton counterparts, clearly have important physiological and cellular differences resulting in differential ecological optimization. Fundamental traits as carbon fixation efficiency, cellular proliferation, and nutrient use need further investigation to better understand *Symbiodinium* community dynamics and predict which coral–*Symbiodinium* associations are at the greatest risk due to climate change.

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