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REPORT

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Patterns of association between *Symbiodinium* and members of the *Montastraea annularis* species complex on spatial scales ranging from within colonies to between geographic regions

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Abstract Patterns of associations between coral colonies and the major clades of zooxanthellae can vary across scales ranging from individual colonies to widely separated geographic regions. This is exemplified in this study of the *Montastraea annularis* species complex from six sites on the Mesoamerican Reef, Belize and nine sites in the Bocas del Toro archipelago, Panama. Restriction fragment length polymorphism (RFLP) analysis of small subunit ribosomal DNA (SSU rDNA) was used to identify the zooxanthellae. In Belize (*M. annularis*), *Symbiodinium* B (79% of the colonies), *Symbiodinium* A, and *Symbiodinium* C were observed. In Panama (primarily *M. franksi*, but also *M. annularis* and *M. faveolata*), there was greater diversity and evenness with *Symbiodinium* A, B, C, C′ (a new symbiont) and D all being common in at least some host/habitat combinations. Non-metric multidimensional scaling ordinations showed that distribution patterns of symbionts across sites are best explained by enclosure (relative influence of open ocean vs. coastal water) and total suspended solids. Because members of clade D are known to be temperature resistant and *Symbiodinium* C′ was found in environments characterized by high sedimentation, these Panamanian reefs may have importance from a management perspective as reservoirs of corals better able to tolerate human impacts.

Keywords Coral · Zooxanthellae · Diversity · Bleaching · Caribbean

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Introduction

Coral reefs are the most diverse and among the most threatened of all marine ecosystems (Bryant et al. 1998; Knowlton 2001; Gardner et al. 2003; Hughes et al. 2003; Pandolfi et al. 2003; Bellwood et al. 2004; Wilkinson 2004). Strategies for conserving coral reefs must focus on both reducing anthropogenic impacts as well as identifying and protecting reefs that are likely to be resilient to stresses that are unavoidable in the short term. The feasibility of the latter approach has grown with the discovery of large amounts of genetic diversity in the symbiotic dinoflagellates (zooxanthellae) with which all reef-building corals associate (Schoenberg and Trench 1980; Rowan and Powers 1991; Rowan et al. 1997; Baker 2003; Knowlton and Rohwer 2003). Evidence exists that some strains of zooxanthellae (as identified by rDNA RFLP patterns) are more resistant than others to environmental stresses (Rowan et al. 1997; Baker 2003; Chen 2003), suggesting that reefs dominated by these relatively stress-tolerant zooxanthellae may have better long-term prospects if properly managed. Consequently, investigating the factors that influence patterns of diversity in coral–algal symbioses on a variety of spatial scales provides information that is highly relevant to coral reef management.

In the tropical western Atlantic, the three members of the *Montastraea annularis* species complex are of particular importance because they are ecologically dominant across a wide array of regions and reef types (Goreau 1959; Weil and Knowlton 1994). They are also known to commonly associate with *Symbiodinium* clades A through D (those clades that regularly associate with

corals) (Rowan et al. 1997; Toller et al. 2001b; Knowlton and Rohwer 2003). Studies of these holobionts (coral hosts and their algal symbionts) have revealed clear differences in distribution that correlate with light, as well as differences in vulnerability to and recovery from coral bleaching (Rowan and Knowlton 1995; Rowan et al. 1997; Toller et al. 2001a, b). These studies also revealed strong differences in zooxanthella communities between near shore and offshore corals (Toller et al. 2001b). Despite differences in light and temperature tolerance, for which the environmental correlates are comparatively clear, it is difficult to tease apart the respective influences of local and larger-scale environmental factors on the mechanisms controlling diversity patterns (Baker 2003). In fact, only a few reefs have been sampled well enough to adequately characterize patterns of zooxanthella diversity.

This study examined the range of spatial scales over which zooxanthella distributions vary within members of the *M. annularis* species complex, and the relative influence of individual environmental factors on the identity of the dominant algal symbiont present at a given spatial scale. To accomplish this, a number of sites were examined in two distinct regions, the Mesoamerican Reef in Belize, and the reefs of the Bocas del Toro region in Panama.

Materials and methods

Study sites

The Mesoamerican Reef in Belize contains the largest barrier reef in the western hemisphere, extending over 250 km, and lies 13–48 km from the mainland. Key features of this system include three offshore atolls and a major lagoon behind the southern portion of the barrier reef. The Mesoamerican Reef is periodically exposed to hurricane disturbance, but generally experiences comparatively little influence from rainfall and land run-off, except in the most southern portion, which is exposed to the major watersheds of Guatemala and Honduras. In contrast, the Bocas del Toro archipelago in Panama is a lagoonal reef system in the semi-enclosed Bahia Almirante, where the reefs are affected by high rainfall and land run-off throughout the year.

Field collections

During July 2002 and August 2003, samples of the dominant *Montastraea* species were collected from Panama's Bocas del Toro archipelago and from Belize's section of the Mesoamerican Reef, respectively. Apparently healthy colonies were sampled using a 1.3 cm steel hole punch and hammer from six sites in Belize and nine sites in Panama (Fig. 1a, b). In Belize, the dominant reef building coral, *M. annularis*, was sampled from back reef (1–3 m) and fore reef (11–14 m) habitats at each site. Tissue samples were preserved in 95% ethanol. In Panama, sites were located in both the inner and outer lagoon habitats at depths of 0–10 m. Samples were taken primarily from the dominant *M. franksi*, though a smaller set of *M. faveolata* and *M. annularis* colonies were sampled for reciprocal comparisons with the Belizean *M. annularis* colonies. The Panamanian *M. annularis* and *M. faveolata* samples were collected from each reef site at which *M. franksi* was sampled and over the same depth range (0–10 m). To further investigate the zooxanthella diversity in this region, *M. franksi* colonies were sampled on both the top and side to characterize microhabitats of relatively high- and low-level irradiance (respectively) within a single colony. Samples were frozen in liquid nitrogen directly after collection.

DNA extractions

DNA was obtained from samples preserved in ethanol using the Dneasy™ Tissue Kit (Qiagen) following the standard protocol. The tissue from frozen samples was thawed and removed from the skeleton using an airbrush and approximately 2 ml of cold deionized freshwater. Blasted tissue was collected in a 200 ml beaker on ice. Skeletal fragments were allowed to settle, and the liquid was transferred to a 2 ml Eppendorf tube. A quarter of the blastate was used for DNA extraction and the rest was archived at −80°C. *Symbiodinium* cells were concentrated in the extraction aliquots by centrifugation at 500*g*. Cells were washed in 1 ml of Zooxanthellae Buffer (ZB) (Rowan and Powers 1991) (0.4 M NaCl, 10 mM EDTA, 20 mM Tris–HCl pH 7.6, 0.05% Tween-20) with a working concentration of 8% 1 M DTT and spun 5 min at maximum speed in a table top microfuge. Samples were then washed with 1 ml ZB without DTT and microfuged for 30 s. Next, they were washed with 1 ml DNA Buffer (DNAB) (Rowan and Powers 1991) (0.4 M NaCl, 50 mM EDTA pH 8) and microfuged for 1 min. DNAB (360 µl) and 10% SDS (40 µl) were added, followed by a 65°C incubation for at least 30 min, and then a Proteinase K lysis (10 µl of 20 mg ml^{-1}) at 37°C overnight or for 3 h at 55°C.

Two 500 µl chloroform extractions were performed followed by 5 min spins at maximum speed. Mussel glycogen (5 µl of 10 mg $ml^{-1}$from Invitrogen) was added as a carrier along with 3 M sodium acetate (10% of the total volume). Two volumes of 100% ice-cold ethanol were added to precipitate the DNA. The samples were placed at −20°C for at least 20 min and then spun at maximum speed for 30 min. A final 70% ethanol wash was followed by 5 min in a speed vacuum at 45°C. The resultant DNA was suspended in 50 µl of water.

A comparison of the two extraction protocols used (frozen and ethanol preserved samples) was performed. The same RFLP results were obtained from a given sample with both extraction methods.

PCR, restriction digests, and sequencing

The SSU rDNA was amplified from each DNA extract using primers ss5 and ss3z (Rowan and Powers 1991)

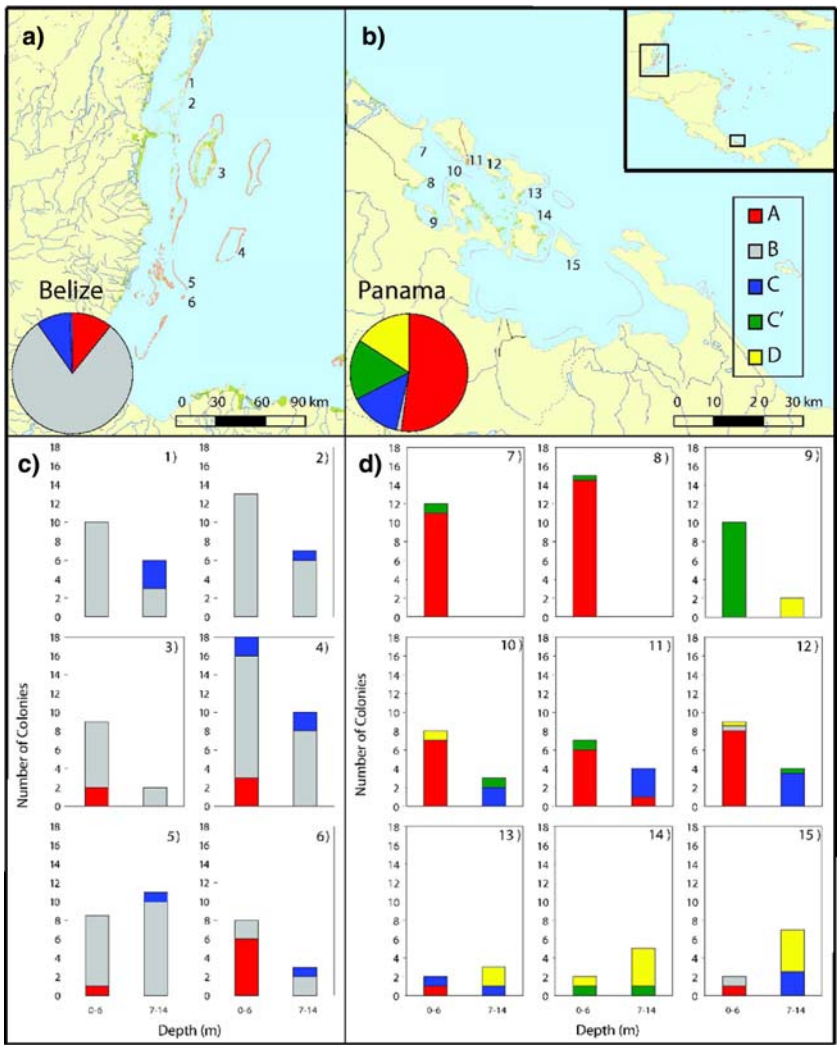

Fig. 1 Sampling locations and zooxanthella distributions by site in **a**, **c** the Mesoamerican Reef, Belize (*Montastraea annularis*, $n = 103$) and **b**, **d** Bocas del Toro, Panama (*Montastraea franksi*, $n = 95$). The pie charts depict the overall relative abundance of each algal symbiont in each region

with the following PCR cycling profile: 2 min initial denaturation at 95°C followed by 45 cycles of 1 min at 94°C, 1 min at 52°C, and 2 min at 72°C. A final extension step for 10 min at 72°C preceded the 4°C hold. The PCR product was digested separately with two restriction enzymes, *Taq*I and *Dpn*II (New England Biolabs), to identify *Symbiodinium* lineages A, B, C, and D following the Rowan and Powers (1991) RFLP protocol.

Cloning and sequencing was performed when necessary to verify identifications based on RFLP. The SSU rDNAs of 11 samples exhibiting a previously unpublished RFLP pattern, referred to henceforth as C′, were cloned using Invitrogen's Sequencing Topo Cloning kit to verify their membership and relative position in the C lineage. These samples had an RFLP pattern that was identical to a *Taq*I digest of *Symbiodinium* C and a *Dpn*II digest of *Symbiodinium* D. This combined RFLP signature was previously only seen in a few experimentally bleached colonies of *Montastraea* (Toller, unpublished) (Fig. 2). Between two and ten clones per sample were sequenced. To verify further the lineages of collected zooxanthellae, the internal transcribed spacer region 2 (ITS2) (330–360 bp fragment) of 80 Panamanian samples (representing all five RFLP-diagnosed genotypes from all habitats in which they were found) were amplified using the LaJeunesse (2002) protocol.

All cloned samples were purified for sequencing using Q-BIOgene's Gene Clean III kit and sequenced using Invitrogen's Big Dye 3.1 following its recommended protocol. Samples were sequenced on an ABI 3100 capillary sequencer, and the data were cleaned and aligned with Sequencher 4.1 software. Alignments were double checked in MacClade.

The SSU rDNA clone sequences were aligned with the sequences obtained by Toller et al. (2001b). The neighbor-joining (NJ) analysis from Toller et al. (2001b) was replicated with the addition of the C′ clones and a bootstrap using Paup 4.0 to determine the phylogenetic relationship between C′ and the other RFLP-distinguishable lineages. A maximum parsimony analysis was performed to verify the NJ results. The ITS2 sequences were aligned in MacClade with all available sequences for each clade compiled by T.C. LaJeunesse (personal communication).

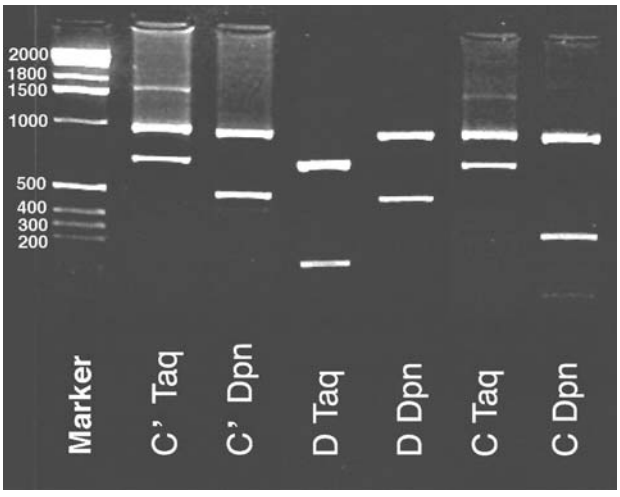

Fig. 2 RFLP Patterns of three *Symbiodinium* SSU rDNA samples. Lane 1 is a marker (base pair sizes indicated), lanes 2 and 3 are the *Taq*I and *Dpn*II digests of *Symbiodinium* C′, 4 and 5 are *Taq*I and *Dpn*II digests of *Symbiodinium* D, and 6 and 7 are *Taq*I and *Dpn*II digests of *Symbiodinium* C. The *Taq*I digests of *Symbiodinium* C′ and C are identical, whereas *Symbiodinium* C′ and D are identical in their *Dpn*II digests. Phylogenetic analysis indicates that C′ is a member of the C clade

Environmental data

Data on six environmental variables (mean depth, temperature, enclosure, total suspended solids, sediment delivery at river mouth per distance to nearest river, and distance to nearest river) were obtained for each site through collection during this study or from previously published studies (Table 1). Actual data were used for depth and temperature (O'Dea and Jackson 2002; Carruthers et al. 2005). Enclosure was calculated as the reciprocal of the sum of the distance to land in eight directions (cardinal and minor) from the site, with 250 km as the maximum distance; as such, it represents the degree to which reefs are influenced by open ocean waters (a low enclosure value) or coastal waters (a high enclosure value). Data on total suspended solids were taken from direct measurements for sites in Panama (Carruthers et al. 2005) and from SeaWIFs images analyzed for sites in Belize (Andrefouet et al. 2002). Sites in Belize were ranked for the level of total suspended solids based on total levels of particulates, chlorophyll *a*, and color dissolved organic matter (the sunlight absorbing portion of dissolved organic matter) because algorithms were not readily available to resolve these components from SeaWIFs images in Type II coastal waters (Andrefouet et al. 2002). Despite the inclusion of chlorophyll *a* and color dissolved organic matter, the values from the SeaWIFs images for Belize were lower at all sites than the total suspended solids for all sites in Bocas del Toro. This allowed for a qualitative comparison across regions. Although it is not possible to account for variation in the relative amount of particulates, chlorophyll *a*, and color dissolved organic matter across sites in Belize, these components are highly positively correlated (Carruthers et al. 2005; D'Sa et al. 2002). Thus, trends in all three components should reflect the trend in total suspended solids and allow for comparisons within Belize. Sediment delivery (a relative measure of the amount of sediment arriving at the river mouth) and distance to nearest river were determined from WRI's Caribbean Reefs at Risk summary erosion statistics for Caribbean watersheds (Burke and Maidens 2004).

Data analyses

To compare the structure of different zooxanthella communities, non-metric multidimensional scaling (MDS) was used. Zooxanthella frequency data were square root transformed before calculating a Bray–Curtis similarity matrix. This moderate transformation allowed less common taxa to contribute more to the similarity index, which is important because the taxa that differed most among samples were the least abundant at some sites. Non-metric MDS was used to ordinate the sites based on the similarity matrix (PRIMER, Clarke and Warwick 1994). The resulting two-dimensional ordination had an acceptable stress level of 0.03 (PRIMER, Clarke and Warwick 1994). The clusters of sites that resulted from this analysis were tested for significance using ANOSIM analysis (PRIMER, Clarke and Warwick 1994).

To determine the extent to which environmental data explained the observed community structures, the ordination of the sites based on zooxanthella data was compared to ordinations of the sites based on all combinations of the environmental variables (BIOENV in PRIMER, Clarke and Warwick 1994). A dissimilarity matrix based on normalized Euclidean distances was calculated for untransformed environmental data for each site. A rank correlation (weighted Spearman) coefficient was computed for comparisons of the biotic similarity matrix and the dissimilarity matrix produced by all potential combinations of the environmental variables. Rank correlations do not require any assumptions about the distribution of the data or the units of measure. The environmental variables that explained the greatest amount of the structure in the biotic ordination were determined by calculating the rank correlation coefficient for every possible combination of environmental variables (from single variables to the total combination of variables). The inclusion of additional variables does not necessarily increase the correlation coefficient, in contrast to multiple linear regression (Clarke and Warwick 1994). This allows for the identification of a limited set of environmental variables that maximizes the correlation between the ordination of the sites by zooxanthella data and by environmental data, while minimizing the stress, or distortion, on the graphical representation. For two-dimensional ordinations, stress < 0.05 is an excellent representation and stress < 0.1 is a good representation with no threat of misleading interpretation (Clarke and Warwick 1994).

Table 1 Environmental variables used for comparison of multi-dimensional scaling ordination of sites by zooxanthellae frequency data and by environmental variables using BIOENV (PRIMER, Clark and Warwick 1994)

Site	Environmental data					
	Mean depth (m)	Temperature (°C)	Enclosure × 10	Total suspended solids (Rank)	Distance to nearest river (km)	Sediment delivery
1B	1.8	30.1	0.073	1	35.82	3.02
1F	12.2	28.5	0.073	1	35.19	3.07
2B	1.8	30.1	0.082	1	24.37	4.43
2F	12.2	28.5	0.082	1	26.31	4.10
3B	1.8	30.1	0.086	2	51.06	22.11
3F	12.2	28.5	0.086	2	51.01	22.13
4B	1.8	30.1	0.097	3	57.54	399.91
4F	12.2	28.5	0.097	4	57.92	397.29
5B	1.8	30.1	0.100	4	40.87	136.80
5F	12.2	28.5	0.101	4	41.92	133.37
6B	1.8	30.1	0.109	4	40.30	76.72
6F	12.2	28.5	0.109	4	41.00	75.41
7	4.0	29.4	2.516	8	16.05	1,906.60
8	3.9	29.5	2.199	9	7.00	4,371.57
9	11.8	30.2	4.543	12	11.48	214.55
10	7.4	28.4	1.657	11	13.14	2,328.84
11	9.6	29.1	0.362	10	16.04	153.55
12	8.2	29.1	6.177	13	20.20	121.93
13	6.6	28.7	0.342	5	29.50	83.49
14	7.0	29.9	0.189	7	31.02	114.09
15	8.6	29.3	1.051	6	18.92	130.18

Results

Molecular analyses

The cloning and sequence data for both the SSU rDNA and ITS2 regions (GenBank accession numbers DQ838542, DQ838543, DQ838544, DQ838545, DQ838546, DQ838547, DQ838548) verified that the genotypes identified by RFLP agreed with those designated by both Rowan and Powers (1991) and LaJeunesse (2001, 2002). Phylogenetic trees including SSU rDNA clones for the previously unpublished C′ genotype placed this variant within the C lineage (bootstrap support >90%; not shown). These samples also constitute a novel type (most closely related to C3) within the known ITS2 diversity for clade C (T.C. LaJeunesse, personal communication). Sequence analysis of all cloned SSU rDNA samples showed some degree of intra-genomic heterogeneity as evidenced by the presence of minor sequence variants across clones from a single sample, and minor bands in RFLP digests and a DGGE gel (see also Toller et al. 2001b).

Regional comparison: Belize versus Panama

The community structure of zooxanthellae harbored by members of the *M. annularis* species complex was significantly different between Belize and Panama ($R = 0.815$, $p < 0.001$) (Fig. 1c, d). In Belize (only *M. annularis* sampled), *Symbiodinium* B dominated the region, associating with 78.6% of colonies sampled ($n = 103$). *Symbiodinium* A and C were also present in 11.7 and 9.7% of colonies, respectively. *Symbiodinium* D and *Symbiodinium* C′ were not found, and only a single clade was detected in any one sample. In Panamanian coral samples, symbiont diversity was both higher and more evenly distributed. In *M. franksi*, *Symbiodinium* A was the most abundant (detected in 51% of the 95 colonies sampled). Three other genotypes observed were represented relatively evenly (13% of colonies contained *Symbiodinium* C, 16% D, and 17% C′), but only 1% of colonies contained *Symbiodinium* B. In only 4% of the colonies was more than one clade detected in a single sample. Much more limited sampling of *M. annularis* and *M. faveolata* also revealed more diversity and more even distributions of symbionts than were observed in Belize (Fig. 3). For example, *Symbiodinium* B was more prevalent in the Panamanian *M. annularis* than *M. franksi*, but *Symbiodinium* D and C′ were also observed, whereas these symbionts were not present in any of the much more numerous Belizean samples of this coral species.

Although Belizean and Panamanian zooxanthella communities differed in their overall structure, similarities in zonation of symbionts with depth existed between the regions. In Belize (Fig. 1c), *Symbiodinium* A was only seen at shallow/back reef habitats, and *Symbiodinium* C was generally found at deep/fore reef habitats ($R = 0.406$, $p < 0.009$). In Panama (Fig. 1d), significant depth zonation differences were exhibited across a continuous reef slope at the individual sites; the frequency of *Symbiodinium* C and D increased with depth, whereas *Symbiodinium* A, B and C′ decreased ($R = 0.428$, $p < 0.003$).

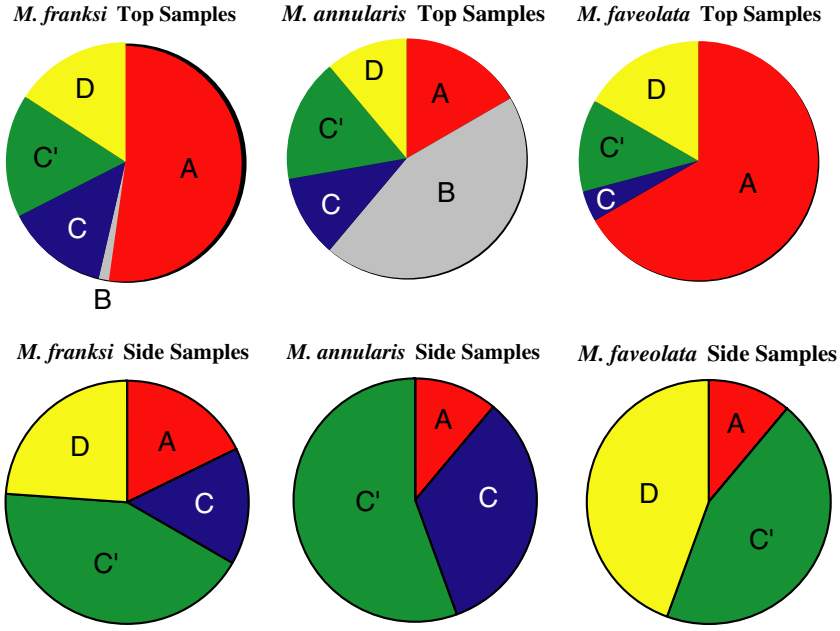

Fig. 3 Pie charts showing the relative abundances of zooxanthellae across the three *Montastraea* species in Bocas del Toro, Panama. The samples sizes are: *M. franksi*, $n = 95$ (*top samples*) and $n = 84$ (*side samples*); *M. annularis*, $n = 9$ (*top samples*) and $n = 9$ (*side samples*); *M. faveolata* $n = 12$ (*top samples*) and $n = 9$ (*side samples*)

Belize exhibited no statistically significant sub-regional variation (Fig. 1c), although *Symbiodinium* A was only found in the southern and atoll sites (#3–6). In contrast, there was a highly significant difference among Panamanian sites that grouped by MDS (Fig. 1d sites 7, 8, 10–12 vs. sites 9, 13–15. $R = 0.75$, $p < 0.008$; MDS analyses presented below). All clades were present in both groupings, but the relative abundances differed greatly. In group 1 (sites 7, 8, 10–12), 77% of colonies associated with *Symbiodinium* A, in contrast to 6% of colonies at sites in group 2 (9, 13–15). *Symbiodinium* C was the next most abundant symbiont at group 1 sites, and the only genotype found in equal proportions in both groups (14%). *Symbiodinium* C′ accounted for only 6% of group 1 colonies' zooxanthellae, whereas 36% of colonies in group 2 associated with it. *Symbiodinium* D accounted for 41% of zooxanthellae at group 2 sites and *Symbiodinium* B represented the remaining 3%. In contrast, 2% and <1% of colonies at group 1 sites associated with *Symbiodinium* D and B, respectively.

Of the Panamanian colonies sampled on both their tops and sides ($n = 78$), approximately half ($n = 40$) had the same genotype in both locations (Fig. 4). More than two-thirds of the corals with a heterogeneous intra-colony distribution of symbionts ($n = 29$) had *Symbiodinium* A on their tops. *Symbiodinium* C′ was sampled from the sides of these colonies more than any other genotype ($n = 20$).

Environmental variables and zooxanthella community structure

Ordination by non-metric multi-dimensional scaling depicts community structure in the zooxanthella data by placing sites with the most similar zooxanthella communities closest to each other (Fig. 5a). MDS depicted Belizean back reef and fore reef habitats as having partially overlapping zooxanthella communities ($R = 0.406$, $p = 0.009$); however, two distinct clusters were derived from the Panamanian sites. These clusters were delineated on the basis of containing outer or inner lagoon sites [although one inner lagoon site (9) clusters with the outer lagoon sites (13–15)] ($R = 0.75$, $p = 0.008$). On the horizontal axis sites cluster by region, whereas on the vertical axis sites cluster by habitats most similar across regions. For example, the cluster representing the four southern-most back reef sites in Belize, which are adjacent to the southern lagoon of Belize and closest to sources of runoff in Honduras and Guatemala, is nearest to the cluster of inner lagoon sites in Panama. The fore reef sites in Belize show no real trend between southern and northern sites but are closer to the outer lagoon sites of Panama than the inner lagoon sites. However, one northern fore reef site (3) and two northern back reef sites (1 and 2) in Belize were found to have all clade B and perfectly overlap in the ordination space. A comparison of the ordination based on zooxanthella data with ordinations based on environmental data had the highest correlation when enclosure alone and enclosure and total suspended solids rank were considered ($\rho_w = 0.524$; ordination shown in Fig. 5b). Of the six environmental variables, enclosure was positively correlated with total suspended solids (0.80) and was negatively correlated with distance to nearest river mouth (−0.58), whereas mean depth and temperature were negatively correlated to each other (−0.77).

Discussion

Members of the *M. annularis* complex within Belize and Panama contain markedly different *Symbiodinium* communities. Compared to the relatively limited number of

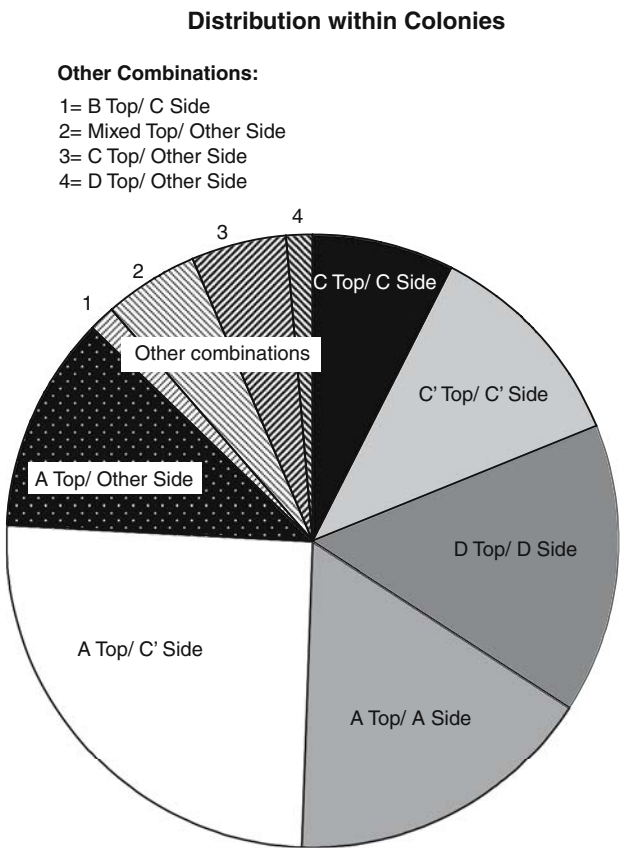

Fig. 4 Top-side zooxanthella distribution within colonies of *M. franksi* ($n = 78$) from Bocas del Toro, Panama (all sites). Of the nine samples that had *Symbiodinium* A on top and a different symbiont on the side, four of these had *Symbiodinium* C, three had *Symbiodinium* D, and two had a mix of *Symbiodinium* A and C′ on the side. The four samples having mixed genotypes on the top and a single genotype on the side were composed of one sample each of the following: *Symbiodinium* B and D on top with *Symbiodinium* A on the side, C and C′ with C, C and D with C, and C′ and A with C′. For the samples with *Symbiodinium* C on the top and a different genotype on the side, one had C′, two had D, and one had a mix of C and D on the side. The one sample that had *Symbiodinium* D on top and a different symbiont on the side was a mix of C and D in the side sample

other sampled western Atlantic sites, Belize represents one of the least diverse zooxanthella communities reported for these corals, whereas Panama represents the most diverse community reported (Table 2). The dominance of *Symbiodinium* B in Belize contributes to the low diversity and evenness associated with the zooxanthella communities in this region. Dominance of *Symbiodinium* B has now been observed at sites across nearly the entire latitudinal range of the tropical western Atlantic and Caribbean [Lee Stocking Island, Bahamas (LaJeunesse 2002), the Mesoamerican Reef, Belize (this study), and offshore reefs of San Blas, Panama (Rowan et al. 1997)]. In contrast, *Symbiodinium* B is rarer on other Panamanian reefs (near shore or lagoonal reefs in San Blas and Bocas del Toro). These observations complicate the interpretation of published reports showing that *Symbiodinium* B dominance within scleractinian corals is positively correlated with high latitude, both in the Caribbean and the Pacific (Baker 1999; Loh et al. 2001; Rodriguez-Lanetty et al. 2001). Although the low temperatures characteristic of high latitude reefs may favor *Symbiodinium* B, our results indicate that multiple environmental factors influence the distribution of *Symbiodinium* B and that sampling should include different environments within latitudinal zones.

Understanding the role of various environmental factors that affect zooxanthella distributions is essential for applying such data to resource management, but there are many difficulties associated with quantifying environmental factors. Few reef sites have environmental data at spatial scales relevant to the short distances over which we observed large differences in symbiont communities in Panama. Also, identification of the most relevant environmental factors may require more controlled field and laboratory experiments.

The zooxanthella distributions observed in Belize and Panama during this study are best explained by two environmental factors: enclosure and total suspended solids. Many small rivers empty into the inner lagoon of the Bocas del Toro archipelago carrying suspended sediments, dissolved organic matter, nutrients, and pollutants. Higher levels of suspended sediments reduce overall irradiance levels. It is likely, then, that irradiance levels at shallower depths in Bocas del Toro may be similar to those found at greater depths in Belize. Diminished irradiance due to fluvial inputs in the outer lagoon of Bocas del Toro may help explain the relatively low abundance of *Symbiodinium* B at these sites as compared with those in Belize.

However, the zooxanthella communities in Bocas del Toro do not simply resemble deeper water Belizean reefs, which largely or entirely lack *Symbiodinium* A, *Symbiodinium* C′, and *Symbiodinium* D. This is not surprising, because fluvial impact may influence the environment in many ways in addition to light penetration. For example, various organic carbons, which are components of land runoff, have been shown to decrease growth and increase mortality in corals (Kuntz et al. 2005; Kline et al. 2006). Similarly, corals have been shown to increase heterotrophic feeding as suspended particulate concentrations increase (Anthony 2000). Thus, the presence of *Symbiodinium* C′ and D and increased abundance of *Symbiodinium* A on the reefs of Bocas del Toro could be due to a variety of mechanisms that were not addressed in this study. *Symbiodinium* A was unusually abundant in experimentally bleached colonies in Panama (Toller et al. 2001a) and thus might be expected to be more abundant in environments subject to disturbance. At several sites, *Symbiodinium* D has been associated with both high and low temperature resistance (Rowan et al. 1997; Toller et al. 2001a; Chen 2003; Baker et al. 2004) and high sedimentation rates associated with deep reefs (Toller et al. 2001b; Chen et al. 2005). Comparable data for *Symbiodinium* C′ are lacking.

The four southern-most back reef sites in Belize show some similarity in zooxanthella communities to the inner

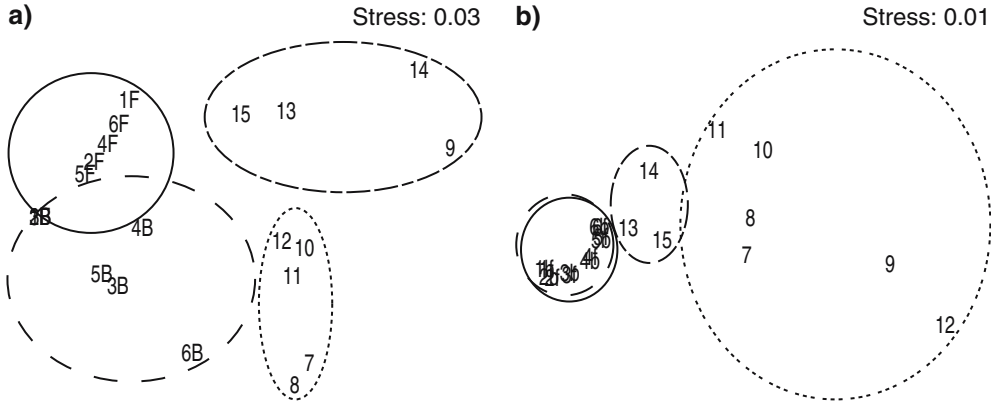

Fig. 5 Clusters of sites identified using non-metric multi-dimensional scaling (*MDS*). Numbers correspond to site numbers in Fig. 1**a**, **b** [#1–6, back reef (*B*) and fore reef (*F*) in the Mesoamerican Reef, Belize (*Montastraea annularis*, $n = 103$) and #7–15 in Bocas del Toro, Panama (*Montastraea franksi*, $n = 95$)]. **a** Non-metric MDS ordination based on the frequency of types of zooxanthellae. **b** Non-metric MDS ordination based on enclosure and total suspended solids (*TSS*) rank. The biologically significant clusters (by ANOSIM analysis: Global $R = 0.78$, $p = 0.01$) are circled in part **a** and **b** in order to visually compare how biological clusters are maintained in the MDS ordination by environmental variables

lagoon sites of Bocas del Toro, Panama, likely because the areas are characterized by similar environmental conditions despite being geographically separated by more than 1,000 km. These sites in Belize have been shown to be periodically influenced by major runoff plumes originating in Honduras and Guatemala (Andrefouet et al. 2002). Interestingly, two of these sites are offshore atolls, which have been assumed to be free from fluvial influence. However, studies of one of the two atolls, Glover's, found unexpectedly high abundances of sponges (Wilkinson 1987) and algae (McClanahan and Muthiga 1998) suggesting that fluvial influence may be higher than assumed. Moreover, the coral community of the southern lagoon of Belize has become more similar to the coral community in Bocas del Toro, Panama in the past twenty years by turning over from an *Acropora*-dominated reef to a reef dominated by *Agaricia tenufolia* and most recently by *Porites furcata* (Aronson et al. 2004).

The abundance of *Symbiodinium* C′ in Bocas del Toro is perplexing in light of the fact that it has not been reported from other regions. The high levels of sediment in the waters of the Bocas del Toro archipelago may create a stressful environment, which may favor unusual zooxanthellae. Site 9 is a particularly unusual reef in that only *Symbiodinium* C′ is found in the shallow region and only stress-tolerant *Symbiodinium* D is found at depth. *Symbiodinium* C′ seems to prefer shallow environments, but is found in relatively lower light environments (colony sides) where the shallow water specialist *Symbiodinium* A also occurs. However, *Symbiodinium* C′ was also found at the deepest sampled areas of sites 10, 12, and 14. Further sampling is needed to determine the extent of the geographic distribution of *Symbiodinium* C′, and experimental studies are required to assess its physiological and competitive abilities.

The molecular data also indicate that finer scale patterns exist beyond those observed at the clade level. Both the SSU rDNA cloning/sequencing and the ITS2 sequencing results showed a higher degree of variability within a given sample than revealed by RFLP. While up to 7% of this variation has been attributed to the intragenomic heterogeneity of SSU rDNA, and possibly PCR artifacts, in another study (Toller et al. 2001b), the level of sequence variability that we observed suggests that the tools we used for distinguishing between genotypes were not sensitive enough to assess fully the variation that may exist. Molecular tools with greater resolution must be adapted and developed to further understand the varying scales of zooxanthella distribution patterns. Examination of this variation in the context of ecological niches and stress histories is equally important to understanding these patterns.

The ability of some corals to host multiple types of zooxanthellae may allow the symbiosis to be more resistant to high temperatures and other aspects of environmental deterioration. Other factors are also important, however. Some corals may routinely host only one kind of zooxanthellae but still be less vulnerable to bleaching either because the zooxanthella hosted is stress resistant (Chen 2003) or because the host itself has genetic characteristics making it less vulnerable to temperature stress. Small-scale environmental variation may also protect some corals, either by minimizing the amount of stress via greater water flow, lower temperatures or shading, or alternatively by inducing host resistance (Brown et al. 2002). The ability to survive and recover from bleaching (e.g. Grottoli 2006) represents an alternative mechanism for coping with stress.

Nevertheless, gaining insight into zooxanthella distributions within an environmental context is an important research priority given the practical applications for coral reef management and conservation (Marshall and Schuttenberg 2005). A significant amount of research remains to be done on the physiological attributes and relative competitive abilities of different zooxanthella

Table 2 Zooxanthella abundances (%), diversity (Simpson's *D*) and evenness (Simpson's *E*) in colonies of *Montastraea* (*ann: annularis, fav: faveolata*, and *fra: franksi*) from all known studies in the Caribbean

Location	Reef type	*Montastraea* species	Depth (m)	Source	Zooxanthellae (%)					*N*	*D*	*E*
					A	B	C	C′	D			
Lee Stocking, Bahamas	Bank barrier	All	4–14	LaJeunesse (2002)	0	73.1	11.5	0	15.4	13	1.75	0.35
		Ann			0	81.3	6.3	0	12.5	8		
		fav			0	60.0	20.0	0	20.0	5		
Mesoamerican Reef, Belize	Barrier/Atoll	*ann*	0–10	This study	11.7	78.6	9.7	0	0	103	1.56	0.31
Bocas del Toro, Panama	Lagoonal	All	1–15	Toller et al. (2001b)	35.7	0	42.9	0	21.4	14	2.80	0.56
		ann			100.0	0	0	0	0	1		
		fav			33.3	0	66.7	0	0	3		
		fra			30.0	0	40.0	0	30.0	10		
Bocas del Toro, Panama	Lagoonal	All	1–8	This study	50.9	4.7	12.5	16.4	15.5	116	2.91	0.58
		ann			16.7	44.4	11.1	16.7	11.1	9		
		fav			66.0	0	4.0	12.5	16.5	12		
		fra			52.1	1.6	13.7	16.8	15.8	95		
San Blas, Panama	Outer fringing	All	0–14	Rowan and Knowlton (1995)	10.2	23.4	66.4	0	0	128	1.98	0.40
		ann			4.2	39.8	56.0	0	0	68		
		fav			22.0	3.4	74.5	0	0	44		
		fra			0	0	100.0	0	0	16		
San Blas, Panama	Outer fringing	All	1–7	Rowan et al. (1997)	16.3	63.8	20.0	0	0	40	2.12	0.42
		ann			4.5	72.7	22.7	0	0	22		
		fav			30.6	50.0	19.4	0	0	18		
San Blas, Panama	Coastal fringing	All	1–12	Toller et al. (2001b)	0	0	31.8	0	68.2	48	1.77	0.35
		ann			0	0	0	0	100.0	4		
		fav			0	0	31.3	0	68.8	24		
		fra			0	0	38.8	0	61.3	20		
San Blas, Panama	Outer fringing	*fra*	4–38	Toller et al. (2001b)	2.6	10.3	80.8	0	6.4	78	1.50	0.30

genotypes. Better knowledge of the factors influencing zooxanthella patterns would provide an important platform for predicting the potential stress responses of the coral host. For example, information regarding the distribution of zooxanthella clades across a region could allow resource managers to consider coral reefs with a high zooxanthella diversity in planning and decision-making procedures. Such data could also inform resource allocation decisions during MPA network design and throughout the adaptive management process. Choosing to protect a variety of habitats with diverse zooxanthella communities may be the best strategy for long-term conservation, since zooxanthella diversity may translate into a greater diversity of coral stress responses, which should lead to more stability over time (Norberg et al. 2001). Lastly, repetitive surveying of an MPA's zooxanthella distributions might alert managers to a change in stress tolerant symbiont abundance. This could facilitate the identification of specific stressors, such as fluvial influence, and motivate local mitigation strategies.

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