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REPORT

Evidence of photoacclimatization at mesophotic depths in the coral-*Symbiodinium* symbiosis at Flower Garden Banks National Marine Sanctuary and McGrail Bank

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Abstract Similar to shallower conspecifics, mesophotic scleractinian corals found at ~ 30–150 m depths maintain important symbioses with photosynthetic microalgae in the genus *Symbiodinium*. Despite the importance of coral-algal symbioses in corals' ability to thrive in multiple dynamic environments and potential role in connectivity, few studies have focused on mesophotic *Symbiodinium* assemblages. This study examines these assemblages in *Montastraea cavernosa* found at shallow (20–25 m) and mesophotic (45–50 m) depths at Flower Garden Banks National Marine Sanctuary and McGrail Bank, in the northwest Gulf of Mexico. Mesophotic corals contained significantly more *Symbiodinium* cells, more chlorophyll *a* per *Symbiodinium* cell, and more chlorophyll *a* and c_2 per unit area coral tissue than shallow corals. However, both mesophotic and shallow *M. cavernosa* contained similar chlorophyll c_2 per *Symbiodinium* cell. Next-generation sequencing of the internal transcribed spacer region (ITS2) of the ribosomal DNA indicated similar *Symbiodinium* assemblage diversity at all banks and between depths. All assemblages were dominated by sequences most closely related to *S. goreaui*, type C1, with three additional low-abundance sequences, identified as 2 C types and 1 A type, also consistently observed among colonies. Both the dominant C1 sequence and the background sequences persisted over two sampling years. These results suggest that algal symbiont assemblages will not limit connectivity potential in *M. cavernosa* in the northwest Gulf of Mexico. Furthermore, we hypothesize that increased *Symbiodinium* abundance may represent an effective light-harvesting strategy on light-limited mesophotic coral reefs.

Keywords Mesophotic · Zooxanthellae · *Symbiodinium* · Gulf of Mexico · Symbiosis · Acclimatization

Introduction

Many species of zooxanthellate corals common to shallow habitats have been recorded in the mesophotic zone, generally considered to range from 30 m to the end of the photic zone (Lesser et al. 2009; Hinderstein et al. 2010). Mesophotic coral ecosystems (MCEs) have drawn increased interest in the past decade for several reasons. Advances in technical diving, mixed gas and rebreather technologies, and the use of remotely operated vehicles have made exploring MCEs safer and more affordable (Pyle 2000). MCEs have also been identified as essential habitat for many commercially and biologically important fish and invertebrate species (Blyth-Skyrme et al. 2013; Lindfield et al. 2016; Loya et al. 2016). Furthermore, the deep reef refugia hypothesis suggests MCEs as refuge for depth-generalist coral species threatened by climate change in shallow environments (Glynn 1996; Bongaerts et al. 2010). However, few studies assessing the validity of the deep reef refugia hypothesis have considered the role algal endosymbionts may play in coral connectivity.

MCEs are characterized by low light, with downward irradiance decreasing exponentially with depth (Lesser et al. 2009). Corals in MCEs experience significantly

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different light spectra than shallow reef conspecifics (Mass et al. 2010). The underwater light field controls rates of primary productivity by *Symbiodinium* spp., influencing coral growth and calcification. One method by which *Symbiodinium* spp. are known to optimize light capture in low-light conditions is by increasing chlorophyll pigment content, both on cellular and areal bases (Dustan 1979; Kaiser et al. 1993; Lesser et al. 2010; Ziegler et al. 2015b). Another pattern that has been observed with increasing depth is decreasing densities of *Symbiodinium* spp. cells within coral tissues (Dustan 1979; Kaiser et al. 1993; Kahng et al. 2010; Ziegler et al. 2015b). It is postulated that decreased densities reduce self-shading (Kaiser et al. 1993; Kahng et al. 2010) and increase light amplification via scattering on coral skeletal features (Marcelino et al. 2013). Additionally, corals may host *Symbiodinium* spp. specialized to low-light environments (Bongaerts et al. 2015a).

Advances in molecular techniques have allowed for new taxonomic descriptions for members within the genus *Symbiodinium* in recent years (Lajeunesse et al. 2012; LaJeunesse et al. 2015; Parkinson et al. 2015). However, because expected levels of diversity within *Symbiodinium* far outnumber existing species descriptions, members of the genus are categorized into nine taxonomic clades, denoted A through I, and are further divided into "types" based on genetic variation below the clade level (Lajeunesse 2002; Correa and Baker 2009; Pochon and Gates 2010; Pochon et al. 2012). Six of the nine clades are known to associate with corals (Baker 2003; Bongaerts et al. 2015b), with many coral species harboring multiple clades or types (Mieog et al. 2007; Silverstein et al. 2012).

Depth zonation of *Symbiodinium* types is common among scleractinian corals, but its occurrence appears to be a function of coral species (Frade et al. 2008; Cooper et al. 2011; Bongaerts et al. 2015b; Ziegler et al. 2015b). Bongaerts et al. (2015b) found that *Stephanocoenia intersepta, Montastraea cavernosa*, and *Madracis pharensis* sampled between 5–60 m showed distinct symbiont profiles at their depth extremes. However, the *Symbiodinium* types observed in *S. intersepta* at 60 m have also been recorded in shallow corals. Additionally, the depth-generalist coral *Agaricia agaricites* associated with a single symbiont type through its sampled range demonstrating that symbiont zonation is not obligatory (Bongaerts et al. 2015b). Similar variations have been seen in depth-generalist coral species in the Pacific Ocean (Chan et al. 2009; Cooper et al. 2011), and the factors that influence the occurrence of symbiont depth zonation are not fully understood. However, if host and symbiont lineages have been coupled through coevolutionary processes, depth- and habitat-related differences in symbiont assemblages may affect coral population connectivity (Loh et al. 2001; Sampayo et al. 2007; Frade et al. 2008; Bongaerts et al. 2010).

Understanding the role symbiont depth zonation plays in distribution of depth-generalist coral species is critical for understanding how MCEs should be managed. Identification of similarities and differences between neighboring shallow and mesophotic reefs is needed to determine whether management plans should treat shallow and mesophotic reefs as separate ecosystems or as a single unit. Offshore banks or seamounts with reefs extending between shallow and mesophotic depths provide ideal sites to study connectivity between adjacent coral populations. The East and West Flower Garden Banks, located in the Flower Garden Banks National Marine Sanctuary (FGBNMS), are two such banks that have been well studied in the northwest Gulf of Mexico (Rezak et al. 1985; Kahng et al. 2010). This study characterizes *Symbiodinium* spp. assemblages in the widespread, depth-generalist coral *M. cavernosa* on adjacent shallow and mesophotic reefs within FGBNMS, as well as on mesophotic reefs of McGrail Bank, a neighboring MCE being considered for inclusion in the sanctuary.

Materials and Methods

Sample collection

FGBNMS is located in the Gulf of Mexico approximately 185 km off the coast of Texas and contains two sampling sites, East Bank (EFGB; 27.91140°N, − 93.59821°W) and West Bank (WFGB; 27.87429°N, − 93.82033°W). The third sampling site, McGrail Bank (27.96364°N, − 92.59216°W), is a MCE, currently designated a Habitat Area of Particular Concern (HAPC), approximately 98 km east of EFGB that has been proposed to be included in boundary expansions of FGBNMS. The depth-generalist coral *M. cavernosa*, common on all sites, has been widely studied in the Caribbean and was selected as the species of interest for this study.

M. cavernosa samples were collected from EFGB, WFGB, and McGrail Bank. At both shallow and mesophotic sites in the FGBNMS, ~ 15 cm^2 coral samples were collected by SCUBA divers using a masonry chisel and hammer. A total of 114 coral samples were collected from FGBNMS, 25 mesophotic (~ 45 m) and 32 shallow (~ 22 m) samples from each bank during October 2014 and 2015. Seventeen additional mesophotic (~ 50 m) samples were collected at McGrail Bank in July 2015. Samples from McGrail Bank were collected using the University of North Carolina at Wilmington (UNCW) *Mohawk* remotely operated vehicle (ROV) and HBOI-designed sampling tool sled. Subsamples used for this study were frozen at − 20 °C until returned to the laboratory on dry ice and then stored at − 20 °C until processing.

As a preliminary assessment of differences between depths and sites, Onset HOBO Pendant loggers were deployed during the October 2015 sampling event. Four loggers, one each at both shallow (20 m) and mesophotic (45 m) locations at EFGB and WFGB, were deployed and collected data every 15 min. Daily maximum, minimum, and average temperatures were calculated. Light data recorded by loggers are excluded from this study because loggers measured lux, which is not an accurate measure of photosynthetically active radiation (PAR).

Symbiodinium density and chlorophyll content

Coral tissue was removed from each sample fragment using a WaterPik™ with sterile Instant Ocean artificial saltwater (IOSW, 35 ppt). Resulting tissue slurries were concentrated via centrifugation and filtered through a 100-µm Nitex mesh screen to remove skeletal fragments and debris. Excess water and any visible animal tissue were removed, and sterile IOSW was added to create final 10-ml *Symbiodinium* sample slurries. Three 2-ml aliquots were taken from each 10-ml sample. Two microliters of 10% formalin were added to one aliquot used for cell counts. Water was removed from a second aliquot and replaced with DMSO-EDTA salt solution to preserve DNA for genetic analysis. The final aliquot was used to determine chlorophylls *a* and c_2 content.

Symbiodinium spp. density (i.e., abundance per cm^2 coral surface) within each sample was determined by performing cell counts on five replicate micrographs, scaled using a hemacytometer (0.1 mm^3), with the program Coral Point Count with Excel extensions (Kohler and Gill 2006). The surface area of the coral fragment was determined using a DAVID SLS-2 structured light 3D scanner. The total number of zooxanthellae cells in each sample was normalized to the fragment surface area to find the number of cells per cm^2 host coral surface area.

Chlorophyll analysis was conducted based on standard procedures within 1 month of sample collection (Jones 1997). A known volume, ranging from 0.5 to 1.0 ml depending on sample viscosity, of each sample was filtered through a 25-mm GF/F filter. Three replicate filters were used for each sample. Filters were frozen overnight to lyse cells and then homogenized in 10 ml of 90% acetone with a tissue homogenizer. Acetone extractions occurred in the dark at 4 °C for approximately 24 h. Sample absorbance at 630, 663, and 750 nm was measured, and these values were used to calculate concentrations of chlorophylls *a* and c_2 using the equations of Jeffrey and Humphrey (1975). Replicates for each sample were averaged, and these averages were used to calculate amount of chlorophylls *a* and c_2 per zooxanthellae cell, amount per cm^2 coral tissue, and the chlorophyll c_2:*a* ratios for each sample.

Symbiodinium genetic identification

An Illumina-based amplicon sequencing method was used to determine *Symbiodinium* diversity within each coral sample (Quigley et al. 2014; Klepac et al. 2015). The internal transcribed spacer 2 (ITS2) region of the ribosomal DNA was chosen for use in identification (LaJeunesse 2001; Lajeunesse 2002; Granados-Cifuentes and Rodriguez-Lanetty 2011). Because the ITS2 region is multi-copy and copy number varies between clades and types (Correa and Baker 2009; Stat and Gates 2011; Stat et al. 2011), sequencing data presented in this study only provide information on relative sequence abundance, not overall zooxanthellae-type abundance.

DNA was extracted from known quantities of approximately 1.5–3 million cells per samples using the Qiagen DNeasy Blood and Tissue Kit. The ITS2 region was amplified via PCR using modified versions of the its-dino forward primer and its2rev reverse primer (Pochon et al. 2001; Stat et al. 2009). Modified primers included a linking sequence, for later addition of index primers, in the form linker+its-dino/its2rev (5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG+GTGAATTGCAGAACTCCGTG-3′; 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG+CCTCCGCTTACTTATATGCTT-3′). Each 30-µl reaction contained 20 ng template DNA, 1 U TaKaRa Ex Taq® HS polymerase, 1× Ex Taq Buffer, 0.2 mM dNTP mixture, and 0.3 µM each forward and reverse primers. The initial denaturing step of 95 °C for 5 min was followed by 25 cycles of 95 °C for 40 s, 65 °C for 2 min, and 72 °C for 1 min. The final extension step was 72 °C for 10 min. If sample showed no band on a 1% agarose gel after the initial 25 cycles, additional cycles were added until a faint band appeared to a maximum of 32 cycles. PCR products were purified using the Thermo Scientific GeneJET Purification Kit.

A second PCR was run to incorporate unique combinations of indexed primers that contained the adapter sequence for Illumina sequencing. Index primer designs were as follows: Illumina adapter sequence + Unique 6 bp Index + Linker (forward and reverse primers, respectively, 5′-AATGATACGGCGACCACCGAGATCTACAC+XXXXXX+TCGTCGGCAGCGTC-3′; 5′-CAAGCAGAAGACGGCATACGAGAT+XXXXXX+GTCTCGTGGGCTCGG-3′). Primers were selected so that each sample had a unique forward and reverse combination (e.g., a unique "barcode"). The 20-µl reaction consisted of 20 ng purified PCR product, 1 U TaKaRa Ex Taq® HS polymerase, 1× Ex Taq buffer, 0.25 mM dNTP mixture, 0.15 µM forward index primer, and 0.15 µM of reverse index primer. The PCR profile for barcode addition was identical to the profile for ITS2 amplification, but only six cycles were necessary to include index primers. Samples were verified on a

1% agarose gel, and band intensity was used to determine the volume of each sample to pool. The pooled sample was purified with the GeneJET Purification Kit and run on a 1% agarose gel stained with SYBR dye. The ~ 500 bp band was excised and soaked in DEPC-treated water overnight at 4 °C to extract the PCR product. Resulting samples were then submitted for Illumina MiSeq sequencing. Samples collected in 2014 were sequenced at the Genomic Sequencing and Analysis Facility at the University of Texas at Austin. Samples collected in 2015 were sequenced at the Scripps Research Institute in La Jolla, CA. Sequencing parameters were identical for both sample sets. A subset of samples from both sampling years was sequenced a second time at Scripps Research Institute to test whether differences seen between sampling years were biological differences or an artifact of sequencing.

Sequencing data were analyzed using an operational taxonomic unit (OTU)-based framework with the clustering program CD-HIT-OTU (Li and Godzik 2006; Fu et al. 2012). Prior to quality filtering with CD-HIT-OTU, the "fastx_trimmer" tool from the FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/index.html) was used to trim all reads to 250 bp, removing a majority of low-quality base calls. Using tools in CD-HIT-OTU, reads with ambiguous base calls, those that did not match the given primer set, or reads that contained > 10 bases with error probability ≥ 0.1 were removed prior to clustering. Paired-end reads were assembled into contigs, allowing up to three mismatches. After filtering and trimming, samples were clustered based on 97% identity. A BLAST search of NCBI's GenBank was used to determine OTU identities.

Statistical analysis

All statistical analysis was done with R v3.2.5. Effects of depth and bank were tested on the dependent factors of zooxanthellae density per cm^2 coral, chlorophyll *a* and c_2 content per zooxanthellae cell and per cm^2 coral, and chlorophyll c_2:*a* ratio. As data did not meet the assumption of multivariate normality and transformations proved insufficient, a permutational multivariate analysis of variance (PERMANOVA) using distance matrices with 999 permutations was conducted with the "adonis" function in the R package vegan (Oksanen et al. 2016). After PERMANOVA revealed differences between depths and banks, univariate analyses of variance (ANOVAs) were conducted for each factor to determine where differences existed. All models also tested for an interaction between depth and bank. To meet univariate normality assumptions, zooxanthellae density and areal chlorophyll content data were log-10 transformed. Because chlorophyll c_2:*a* data were negatively skewed, a Box–Cox power transformation was used to determine that the ratios met normality assumptions when transformed by the −4th power (Box and Cox 1964). Cellular chlorophyll *a* and c_2 did not require transformation.

OTU count data were analyzed using the R package MCMC.OTU (Green et al. 2014). Prior to modeling, samples with low read abundance, based on a default z-score cutoff of − 2.5, were removed. Based on these parameters, one shallow and one mesophotic sample each from EFGB and WFGB were excluded from the full analysis, and an additional mesophotic sample from WFGB was excluded from the mesophotic-only analysis. The function "mcmc.otu" was used to infer changes in relative proportions of the identified OTUs, testing the fixed effects of bank, depth, and the interaction between bank and depth. McGrail was excluded in the model testing for effects of depth because no shallow samples were collected from this bank. Separate models were run to compare the mesophotic assemblages among the three sampled banks and to test for effects of sampling year within East and West Bank. The same analyses were performed on the data from samples sequenced a second time in order to determine whether differences between years were an artifact of separate sequencing.

Divergence between the OTU sequences was evaluated with a haplotype network in the R package Pegas (Paradis 2010). OTU sequences were aligned and trimmed to be of equal length, with gaps coded such that each indel was considered a single point mutation. Sequences of *Symbiodinium* type C1 (*S. goreaui*) and three closely related variants taken from SymbioGBR (Tonk et al. 2013) were included in the network. The function "mst" was used to create a minimum spanning network based on base pair differences.

Results

Environmental gradients at FGBNMS

Mean temperature differed across depths (ANOVA; $F = 8.729$, $p < 0.001$), but not between banks (ANOVA; $p > 0.05$). Temperatures difference between depths averaged 0.25 °C, representing an approximately 1% decrease from shallow to mesophotic.

Symbiodinium spp. density and chlorophyll content

The suite of *Symbiodinium* spp. response variables in this study differed by both depth (PERMANOVA, $F = 69.661$, $p < 0.001$) and bank (PERMANOVA, $F = 9.790$, $p < 0.001$). There was no significant interaction between bank and depth. *Symbiodinium* spp. density per unit area coral in mesophotic *M. cavernosa* colonies was

significantly greater than in shallow colonies ($F = 24.109$, $p < 0.001$). Mesophotic corals contained an average of 440,252 more symbiont cells per cm^2 than shallow corals, representing an approximately 25% difference. *Symbiodinium* spp. densities also differed among colonies sampled from different banks ($F = 8.746$, $p < 0.001$). Post hoc Tukey tests revealed that McGrail contained more *Symbiodinium* spp. cells than EFGB, which did not differ significantly from WFGB (Fig. 1).

Chlorophyll *a* and c_2 content, in picograms per *Symbiodinium* cell, differed between banks (*a*: $F = 5.908$, $p < 0.004$; c_2: $F = 5.427$, $p < 0.006$), with EFGB and WFGB containing similar amounts and McGrail containing higher chlorophyll concentrations per cell (Fig. 2a). Mesophotic samples contained more cellular chlorophyll *a* than shallow samples ($F = 7.890$, $p < 0.006$), but chlorophyll c_2 was not significantly different between shallow and mesophotic. There were no significant interactions between depth and bank for chlorophylls *a* or c_2.

Overall chlorophyll content per unit area coral was higher in mesophotic samples than shallow samples (chl *a*: $F = 50.348$, $p < 0.001$; chl c_2: $F = 34.042$, $p < 0.001$). Areal chlorophyll *a* and c_2 both differed between all three banks (chl *a*: $F = 22.873$, $p < 0.001$; chl c_2: $F = 24.221$, $p < 0.001$), with the highest levels seen at McGrail and lowest levels at EFGB (Fig. 2b). There were no significant interactions between bank and depth. Ratios of chlorophyll c_2:*a* were lower in samples from mesophotic depths ($F = 17.856$, $p < 0.001$). Differences also existed between banks ($F = 4.276$, $p < 0.02$; Fig. 2c).

Symbiodinium clade type identification

Of the 114 total samples collected from FGB, 99 were successfully amplified and submitted for sequencing, including 62 shallow and 37 mesophotic samples. Eleven of the 17 McGrail samples were successfully amplified and submitted for sequencing. In total, 5,664,259 aligned sequences were mapped to four reference OTUs. Average length of the OTUs was 341 bases, ranging from 313 bases (OTU 3) to 402 bases (OTU 1). The number of sequences per sample mapped to each OTU ranged from 0 to 254,365, averaging 12,991 reads per OTU per sample. OTU 1 was the dominant OTU in all samples with the exception of two outliers, which were dominated by reads from OTU 3.

Nucleotide sequence queries run in the NCBI GenBank blastn suite matched OTU 1 to *Symbiodinium* type C1.168 (EU786002, Fay et al. 2009) with 100% identity, which differs from the consensus sequence for *S. goreaui* (type C1) by a single base. Similarly, OTU 3 matched *Symbiodinium* type A13 (JN558094, Pochon et al. 2012) with 100% identity, differing from the consensus ITS2 sequence of *S. microadriaticum* (type A1) by one base (LaJeunesse 2001). The remaining OTUs were identified as most similar to sequences in clade C. OTU 2 had 92% sequence similarity to other C1 variants previously reported by Fay et al. (2009). OTU 4 matched a C variant found in a corallivorous flatworm with 99% identity (Hume et al. 2014). The three OTUs identified as clade C variants differed by between 8 and 26 base pairs, with all differences due to a single indel except for one nucleotide in OTU 4 that differed from OTUs 1 and 2 (Fig. 3).

The R package MCMC.OTU was used to analyze sequence count data. In total, OTU 1_C1.168 represented 91.4% of total sequence reads. The remaining three OTUs comprised only 2.9% (OTU 2_C), 2.8% (OTU 3_A13), and 2.9% (OTU 4_C) of total reads. All OTUs were present in all samples with the exception of OTU 3, which was only present in 30.5% of the samples (Fig. 4).

Samples from McGrail Bank were excluded from models comparing bank and depth due to the lack of shallow habitat at McGrail Bank. OTU 3_A13 could not be modeled reliably in this comparison due to the outlier samples dominated by this OTU. For the remaining OTUs, after *p* values were adjusted for multiple comparisons, differences were only found between proportions of OTU 2_C and OTU 4_C. However, data from repeat sequencing of a subset showed no differences between depths or banks, indicating that differences in background OTUs resulted from sequencing bias introduced by sequencing at different facilities. A model comparing shallow and mesophotic assemblages with East and West FGB that included only samples collected in 2015 showed that no difference existed between depths.

To determine whether differences existed between all three banks, separate analysis was run for mesophotic samples from EFGB, WFGB, and McGrail Bank. Shallow samples were excluded because McGrail Bank lacked shallow samples, creating an unbalanced design. OTU 3_A13 was excluded because it was present in less than 10% of samples. No OTUs showed significant differences in proportions between banks. Separate analysis was conducted to determine whether shallow assemblages differed between EFGB and WFGB. Two samples, one from each bank, were excluded from analysis due to low read counts. Only OTU 3_A13 differed between banks, with higher proportions at EFGB due to the shallow outlier sample dominated by this OTU.

Discussion

Symbiont density is an integral determinant of holobiont physiology and a mediator of symbiosis function (Cunning and Baker 2014; Cunning et al. 2015; Scheufen et al. 2017). The ability of corals to photoacclimatize to low

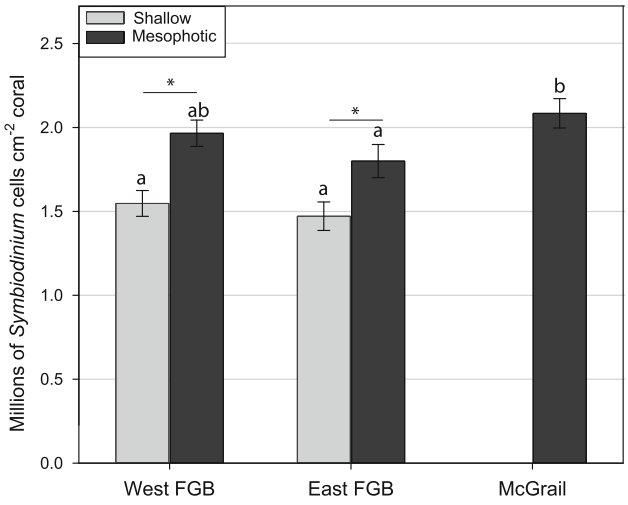

Fig. 1 Mean density ± SE of *Symbiodinium*, in millions of cells cm^{-2}, among *M. cavernosa* from shallow and mesophotic depths at West Flower Garden, East Flower Garden, and McGrail Banks. Significant differences between depths are indicated by an asterisk (*), and pairwise differences between sites are indicated by letters

irradiance is mainly a function of the coral's resident *Symbiodinium* spp. and their photosynthetic apparatus organization (Lesser et al. 2010; Bongaerts et al. 2015b; Ziegler et al. 2015a; Scheufen et al. 2017). Here, we demonstrate that *M. cavernosa* found on three mesophotic reefs in the Gulf of Mexico exhibits increased densities of *Symbiodinium* spp. cells within their tissues as compared to shallow water conspecifics in the region. This increase in *Symbiodinium* spp. cells corresponds to an overall increase in light-harvesting chlorophyll pigments within the coral. No other studies have reported significantly increased *Symbiodinium* densities in scleractinian corals from shallow to mesophotic to date.

In contrast to studies suggesting decreased zooxanthellae densities with increased light-harvesting pigments per cell (Dustan 1979; Kaiser et al. 1993; Kahng et al. 2010), the overall increases in chlorophyll *a* and c_2 pigments in the coral tissues seen here were caused by a combination of increased zooxanthellae densities in the tissues and increases in cellular chlorophyll. A previous study of *M. cavernosa* in the Bahamas found no differences in zooxanthellae densities over a depth gradient of 3–91 m (Lesser et al. 2010). However, patterns of increasing *Symbiodinium* with decreasing light intensity have been reported in different contexts. Negative correlations between light intensity and symbiont density have been reported at shallow depths in *Acropora globiceps* (Ladriére et al. 2014) and *Stylophora pistillata* (Falkowski and Dubinsky 1981). Experimental shading of the coral *S. pistillata* resulted in immediate increases in both pigment content and symbiont cell densities caused by changes in rates of cell division (Titlyanov et al. 2001). Similarly, *Symbiodinium* densities

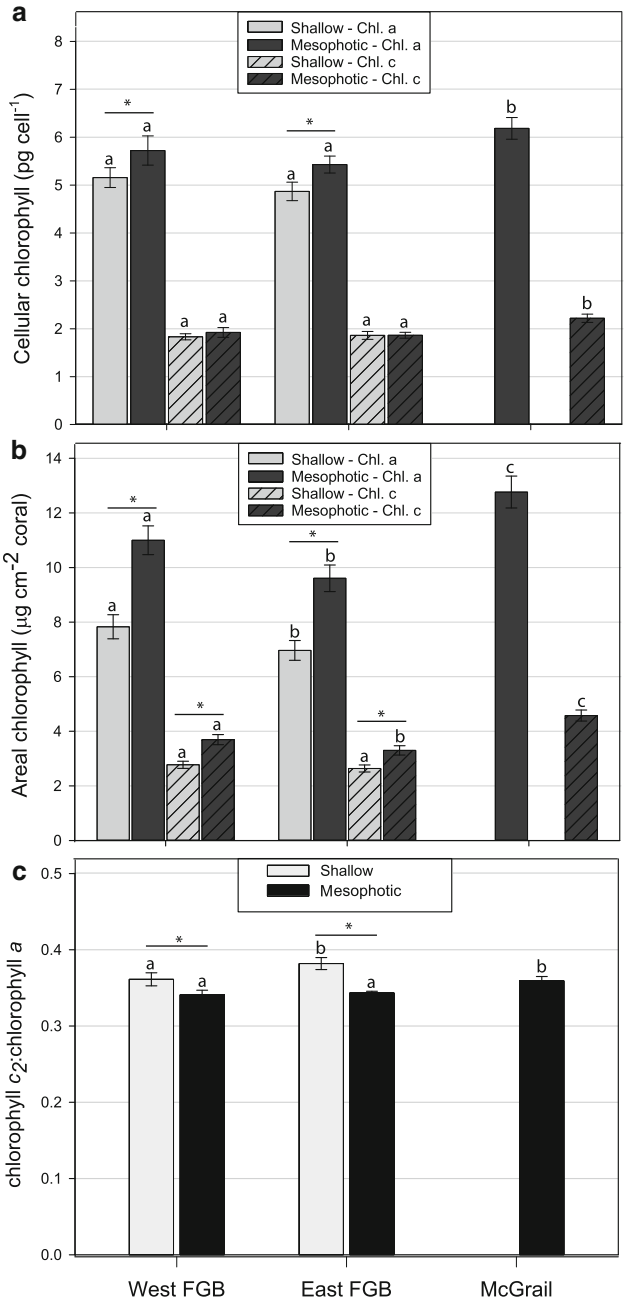

Fig. 2 **a** Mean ± SE chlorophyll *a* and c_2 content, in picograms, per *Symbiodinium* cell from shallow and mesophotic samples at each of the three banks. **b** Average chlorophyll *a* and c_2 content, in micrograms, per square centimeter coral surface area. **c** Average ratio of chlorophyll c_2 to chlorophyll *a* in shallow and mesophotic samples from each of the three banks. Significant differences between depths are indicated by an asterisk (*), and pairwise differences between sites are indicated by letters

in anemones have been shown to increase under low-light experimental conditions (Saunder and Muller-Parker 1997). Seasonal variation in symbiont density, with the highest densities seen in winter and spring when solar radiation is weaker, has also been widely reported

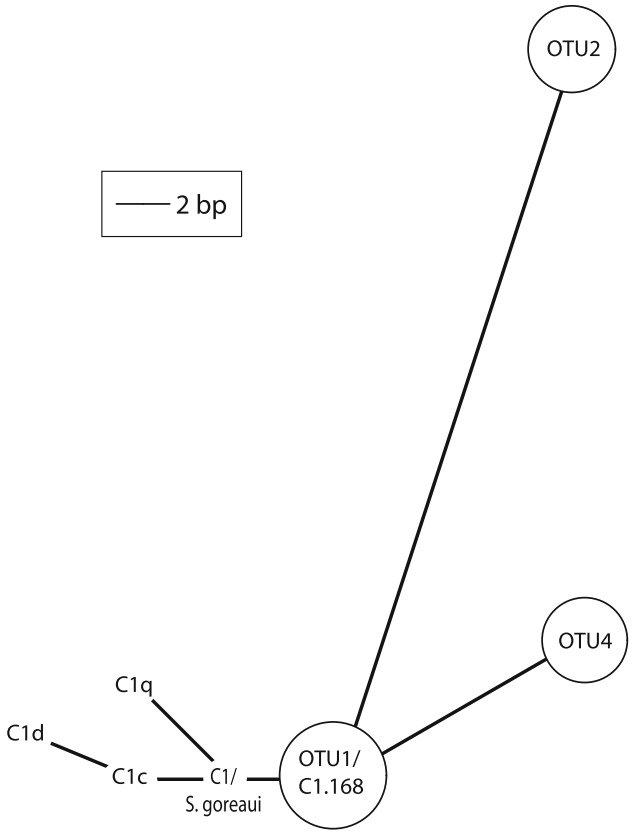

Fig. 3 Minimum spanning network of *Symbiodinium* internal transcribed spacer 2 (ITS2) diversity for C types seen at Flower Garden Banks National Marine Sanctuary (FGBNMS) and McGrail Bank and four known variants within clade C. Circles represent sequences found in this study

(Fagoone et al. 1999; Fitt et al. 2000; Thornhill et al. 2011; Chauka et al. 2015). We hypothesize that the observed increases in *Symbiodinium* spp. densities among mesophotic *M. cavernosa* at Flower Garden Banks represent photoacclimatization to lower light availability at mesophotic depths.

Increased heterotrophic feeding and therefore increased nitrogen availability at mesophotic depths could also be partially responsible for increases in zooxanthellae division rate (Cook et al. 1988; Dubinsky et al. 1990; Marubini and Davies 1996; Ferrier-Pagès et al. 2003; Jamieson et al. 2007; Alamaru et al. 2009; Houlbrèque and Ferrier-Pagès 2009). Lesser et al. (2010) identified a transition from net autotrophy to heterotrophy between 46 and 61 m. However, the present analysis did not include *M. cavernosa* colonies sampled beyond 60 m, as reef habitat at sampling sites in FGBNMS did not extend to that depth. Alternatively, symbiotic cyanobacteria can act as a nitrogen source for *Symbiodinium* spp., and their occurrence in *M. cavernosa* has been positively correlated with colony depth (Lesser et al. 2007). Measurements of photosynthetic rates and efficiency, potentially coupled with isotope analysis, could provide further insight into the causes and physiological effects of increased zooxanthellae densities among mesophotic *M. cavernosa* at the Flower Garden Banks and McGrail Bank.

Decreased ratios of chlorophyll c_2:*a* conflict with the hypothesis of Kaiser et al. (1993) that zooxanthellae will increase chlorophyll c_2 with depth in order to photoadapt to

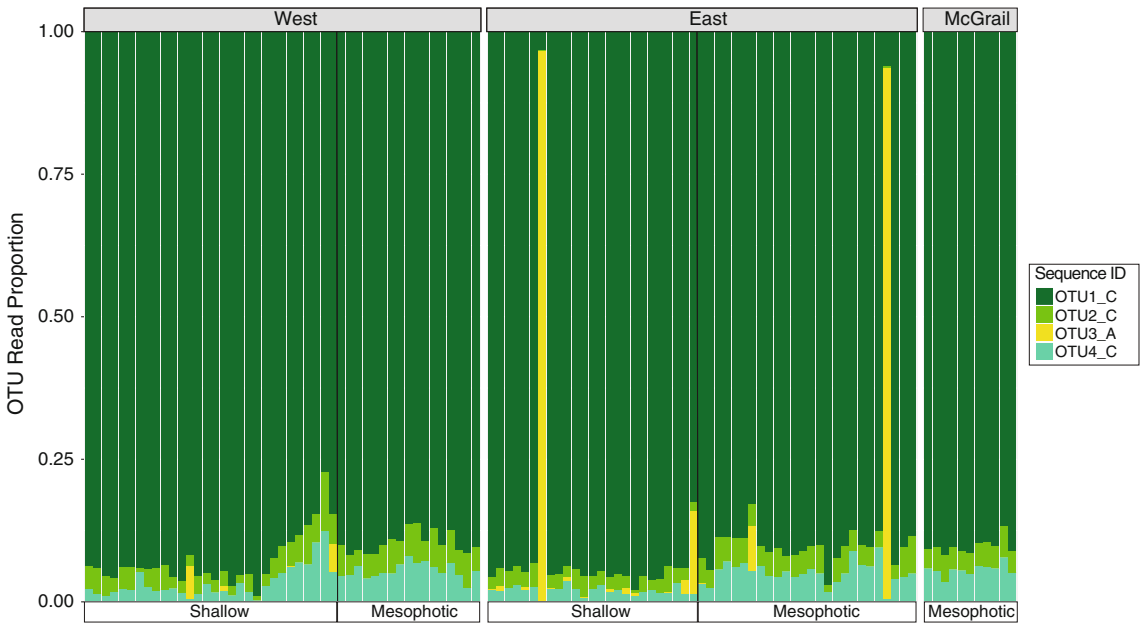

Fig. 4 Sequence read proportions of the four identified *Symbiodinium* operational taxonomic units (OTUs) seen in each sample at East Flower Garden Bank, West Flower Garden Bank, and McGrail Bank

blue light. However, wavelength transformation of blue light to orange-red light by host-expressed fluorescent protein pigments has been observed at mesophotic depths (Smith et al. 2017) and would explain increased chlorophyll *a* pigment in zooxanthellae. Lower c_2:*a* ratios have also been observed at mesophotic depths in *M. cavernosa* in the Bahamas, *Seriatopora hystrix* in the Gulf of Eilat, and *Leptoseris* spp. in Hawaii (Lesser et al. 2010; Nir et al. 2011; Kahng et al. 2012).

Similar *Symbiodinium* assemblage diversity between depths despite differences in light and temperature suggests that corals in this study do not utilize depth-specialist symbionts in order to acclimatize to mesophotic depths, providing further support to the notion of increased zooxanthellae densities as a strategy to optimize light capture. These results are similar to another study which found that *M. cavernosa* sampled at $\leq$ 10 m, 15–20 m, and $\geq$ 25 m depths in the Florida Keys, Bermuda, and US Virgin Islands associated with the same dominant *Symbiodinium* type, C3, throughout the sampled range (Serrano et al. 2014). In contrast, other studies of zooxanthellae assemblages in *M. cavernosa* have observed patterns of zonation (Lesser et al. 2010; Bongaerts et al. 2015b). However, in both of these studies, all were closely related variants within clade C, most closely related to type C3 (Lesser et al. 2010; Bongaerts et al. 2015b). Additionally, Lesser et al. (2010) did not observe this shift until a depth of 60 m. Since the maximum depth sampled in this study was 50 m, shifts to alternative *Symbiodinium* types could be possible among *M. cavernosa* $>$ 60 m on other reefs in the Gulf of Mexico.

It is possible that sequence diversity seen within this study underestimates *Symbiodinium* species diversity found in *M. cavernosa* in the northwest Gulf of Mexico, as analyses with different genetic markers have shown that genetically distinct lineages can possess identical ITS sequences (Santos et al. 2004; LaJeunesse and Thornhill 2011; Thornhill et al. 2013). Conversely, the three OTU sequences identified as C variants could represent a single *Symbiodinium* spp., as previous sequencing of isoclonal cultures identified high levels of ITS2 sequence diversity within members of the same species (Thornhill et al. 2007; LaJeunesse and Thornhill 2011; Arif et al 2014). Despite difficulties in using ITS2 as sole means of identification, ITS2 genotyping acts as an informative proxy for assessing *Symbiodinium* diversity (Sampayo et al. 2009; LaJeunesse and Thornhill 2011). In this study, genetic distances between the four ITS2 C variants are similar to those seen by Arif et al. (2014) in ITS2 sequences from isoclonal cultures of C1. This, coupled with the fact that most sequence differences were caused by single indels and all C variants were present in all samples, leads the authors to hypothesize that low-abundance C variants seen in this study likely represent intragenomic variation within a single dominant C1 type. Sequencing of additional genetic markers among *Symbiodinium* could address this hypothesis.

With respect to the dominant *Symbiodinium* ITS2 sequence observed, this study concurred with previous observations that *M. cavernosa* predominantly associates with types in clade C (Lajeunesse 2002; Savage et al. 2002; Correa et al. 2009; Finney et al. 2010; Serrano et al. 2014; Bongaerts et al. 2015b; Klepac et al. 2015). However a majority of the previous studies found assemblages dominated by genetic variants within *Symbiodinium* type C3 (Lajeunesse 2002; Correa et al. 2009; Finney et al. 2010; Serrano et al. 2014; Bongaerts et al. 2015b). In this study, all assemblages, with the exception of two outliers, were dominated by sequences identified as most closely related to *S. goreaui* (type C1). While *S. goreaui* (C1) has been reported in association with *M. cavernosa* previously, it was only observed in low proportions within a subset of shallow (15 m) samples (Lesser et al. 2010). *M. cavernosa* obtains its endosymbionts through horizontal transmission, and therefore it is possible that differences in dominant C type could be due to differences in the environmental pool of available zooxanthellae.

This study presents the first observation of evidence for a strategy by which the depth-generalist coral *M. cavernosa* optimizes productivity and survivorship at mesophotic depths in the Gulf of Mexico. By hosting more algal symbionts within their tissues, the mesophotic corals increase the amount of light-harvesting pigments and thus increase their photosynthetic capacity. Additionally, similar zooxanthellae assemblages across depths and banks suggest that the potential for both vertical and horizontal connectivity among *M. cavernosa* populations in the northwest Gulf of Mexico is not restricted by *Symbiodinium* associations. Similar assemblage makeup across depths and banks also suggests that the different banks and depth zones at Flower Garden Banks National Marine Sanctuary and the surrounding region can be considered a single holobiont type when developing management strategies.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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