Linking photoacclimation responses and microbiome shifts between depth-segregated sibling species of reef corals

Carlos Prada[1*+], Tomás López-Londoño[2+], F. Joseph Pollock[2,3], Sofia Roitman[2], Kim B. Ritchie[4], Don R. Levitan[5], Nancy Knowlton[6], Cheryl Woodley[7], Roberto Iglesias-Prieto[1], Mónica Medina[1]

[1]*University of Rhode Island, Department of Biological Sciences, Kingston, RI 02881, USA*
[2]*Pennsylvania State University, Department of Biology, 208 Mueller Lab, University Park, PA 16802, USA*
[3]*The Nature Conservancy, Hawai'i and Palmyra Programs, 923 Nu'uanu Avenue, Honolulu, HI 96817, USA*
[4]*University of South Carolina Beaufort, Department of Natural Sciences, 801 Carteret Street, Beaufort, SC 29906, USA*
[5]*Florida State University, Department of Biological Science, Tallahassee, FL 32306, USA*
[6]*National Museum of Natural History, Smithsonian Institution, Washington, DC 20560, USA*
[7]*National Oceanic and Atmospheric Administration, National Ocean Service, National Centers for Coastal Ocean Sciences, Hollings Marine Laboratory, Charleston, SC 29412, USA.*
[*]*Corresponding author: prada@uri.edu.*
[+]*Equal contribution.*

ABSTRACT

Metazoans host complex communities of microorganisms that include dinoflagellates, fungi, bacteria, archaea, and viruses. Interactions among members of these complex assemblages allow hosts to adjust their physiology and metabolism to cope with environmental variation and occupy different habitats. Here, using reciprocal transplantation across depths, we studied adaptive divergence in the Caribbean corals *Orbicella annularis* and *O. franksi*. When transplanted from deep to shallow, *O. franksi* experienced fast photoacclimation, low mortality, and maintained a consistent bacterial community. In contrast, *O. annularis* experienced higher mortality, and limited photoacclimation when transplanted from shallow to deep. The photophysiological collapse of *O. annularis* in the deep environment was associated with an increased microbiome variability and reduction of some bacterial taxa. Differences in the symbiotic algal community were more pronounced between coral species than between depths. Our study suggests that these sibling species are adapted to distinctive light environments partially driven by the algae photoacclimation capacity and the microbiome robustness, highlighting the importance of niche specialization in symbiotic corals for the maintenance of species diversity. Our findings have implications for the management of these threatened Caribbean corals and the effectiveness of coral reef restoration efforts.

Keywords: corals, symbiosis, microbiome, photobiology, ecophysiology, niche divergence

INTRODUCTION

Understanding how microbial biodiversity interacts with their host's physiology is essential for understanding animal ecology and evolution (Thompson et al. 2015). Microbial communities often fine-tune their host's physiology to cope with environmental variation across habitats (Gilbert et al. 2010). Reef-building corals (Cnidaria: Scleractinia) form a symbiotic association with dinoflagellates, which allow corals to thrive on the ocean's euphotic zone along a strong depth-mediated light gradient (Stoddart 1969). Corals living at different depths possess distinctive physiological and morphological traits to optimize energy acquisition which results from genotypic and phenotypic variation within and between coral species (Vermeij and Bak 2002; Hoogenboom et al. 2008). Coral colonies at different depths may host distinctive symbiotic algae with contrasting photoacclimation capabilities that grant their hosts the ability to thrive in certain light environments (Rowan et al. 1997; Warner et al. 2006). Because of these differences in photoacclimation and the prevalence of specific associations with coral hosts, zonation by light has been regarded as a primary form of niche partitioning in symbiotic corals (Iglesias-Prieto et al. 2004).

While the influence of different species of symbiotic algae on the ecophysiology of reef-building corals has been studied, the effect of other coral-associated microorganisms is less known, specially across depth-segregated species (Rohwer et al. 2002; Pantos et al. 2015). However, the interest on coral-associated microbes and their roles in maintaining health and preventing diseases has increased substantially (Kellogg et al. 2013; Peixoto et al. 2017). From an eco-evolutionary perspective, the evidence suggests that coral-associated bacterial assemblages can be highly variable although "footprints" of unique microbial assemblages appear to be mediated by a combination of host species and local environmental conditions (Rohwer et al. 2002; Marchioro et al. 2020). These patterns indicate that bacterial communities, like photosynthetic dinoflagellates, could also be spatially structured and segregated along environmental gradients.

Recently diverged coral species that differ in their vertical distribution are ideal systems to study the microbiota-animal relationship as a potential basis for habitat specialization. The *Orbicella* species complex, dominant in Caribbean reefs, was initially regarded as one species with ecotypic variation, but recent research revealed three species partially segregated by depth (Weil and Knowlton 1994; Fukami et al. 2004; Levitan et al. 2011). *O. annularis* is a high-light specialist which forms columns with senescent edges, while *O. franksi* is a low-light specialist forming irregular mounds and plates. *O.*

faveolata form massive mounds and can overlap with both *O. annularis* and *O. franksi* habitats. The three *Orbicella* species are closely related with incomplete lineage sorting across nuclear and mitochondrial markers (Weil and Knowlton 1994; Fukami et al. 2004). The symbiotic dinoflagellate communities (Rowan et al. 1997; Kemp et al. 2015) as well as the photobiology of this species complex have been extensively studied (Warner et al. 2006; Scheufen et al. 2017), enabling the identification of important differences mediated by environmental gradients. The *Orbicella*-associated bacterial communities have also been examined (Kellogg et al. 2013; Roitman et al. 2020). Therefore, this coral species complex offers an ideal system for the study of how species specialize to live in different habitats through adaptive divergence.

Using a reciprocal transplant experiment between shallow and deep environments in Bocas del Toro (Caribbean Panama), we studied adaptive divergence between the youngest sister species within the *Orbicella* species complex, *O. annularis* and *O. franksi*. We surveyed colonies for survivorship and characterized the algal symbiont and microbial communities across habitats. We also evaluated if these recently diverged species have also diverged physiologically along depth-mediated light gradients. We hypothesize that *O. franksi* and *O. annularis* exploit different light niches, coexisting in Caribbean reefs with minimal competition for space. Our findings suggest that despite being so young (< 500K) (Pandolfi et al. 2002), these two sister species have diverged and fine-tuned their photoacclimation capabilities and microbial symbionts to maximize efficiency in their own light environments.

MATERIALS AND METHODS

Reciprocal transplantation

To study the effects of depth and light in *O. annularis* and *O. franksi,* colonies were reciprocally transplanted between shallow and deep environments at Bocas del Toro, Panama (latitude: 9.327222, longitude: -82.203889). The study site is located on the slope of a relative narrow reef protected on all sides by islands and has been monitored for coral spawning for two decades (Levitan et al. 2011). This location is ideal to study adaptation across depths because the vertical distribution of these species is compressed to shallower depths (~2-9 m) compared to other sites in the Caribbean (Van Veghel 1994; Pandolfi and Budd 2008), although maintaining the typical vertical zonation pattern (*O. annularis* in shallow-water and *O. franksi* in deeper-water).

In September 2014, fully pigmented coral clonemate fragments (~5 cm in diameter) were collected from the edges of *O. franksi* colonies and vertically oriented colonies of *O. annularis*. Same genotypes (clonemates) of both species ($n \leq 28$) were exposed to both shallow and deep environments. Coral fragments were collected from two depths in which each species was abundant: shallow for *O. annularis* (3–4 m) and deep for *O. franksi* (7–8 m). Coral fragments from each species were transplanted to polyvinyl chloride (PVC) panels placed near the original depth of collection (3.5 m and 9.5) where they were left to heal and acclimate for one week. Subsequently, *O. annularis* colonies were transplanted from shallow to shallow (S-S) ($n = 27$) and shallow to deep (S-D) ($n = 30$). Similarly, *O. franksi* colonies were transplanted from deep to shallow (D-S) ($n = 44$) and deep to deep (D-D) ($n = 28$).

To test for differential mortality across depths, we visually inspected colonies six months after transplantation in March 2015. One detached individual from *O. annularis* transplanted deep was discarded from this analysis. A one-tailed Fisher exact test was used to assess differences in survivorship among sites. To standardize the fitness (*i.e.,* survival) advantage on the original depth over the opposite depth for each species, differences in fitness were divided over the average fitness on each particular habitat (Hereford 2009).

Samples were collected in accordance with local regulations under CITES permits PWS2014-AU-002155 and 12US784243/9 and Panama permit number SE/A-94-13.

Environmental parameters

To characterize the effect of the water optical properties on light availability across depths, we measured the diffuse attenuation coefficient for downwelling irradiance (K_d) at the beginning of the experiment. K_d was calculated by measuring changes in light intensities across the depth gradient using the cosine-corrected PAR sensor of a Diving-PAM (Walz), previously calibrated against a manufacturer-calibrated quantum sensor (LI-1400, LI-COR). The light intensity at each transplant site, expressed as the percentage of incident light, was calculated (Kirk 2011; López-Londoño et al. 2021). Variation in temperature and relative light levels throughout the duration of the experiment was recorded every 30 min by Onset HOBO data loggers (UA-002-64, Onset Computer Corporation) attached to the PVC panels.

Photophysiology

To test how depth-dependent light variation affects the photosynthetic condition of corals' symbiotic algae, we measured the chlorophyll *a* (Chl *a*) fluorescence using pulse amplitude modulated (PAM) fluorometry (Diving-PAM). Measurements were recorded on ten fragments from each species at each depth before transplantation, and every two/three days during the week after transplantation. The effective quantum yield ($\Delta F/F_m'$) of photosystem II (PSII) was recorded at noon during peak sunlight exposure, and the maximum quantum yield of PSII (F_v/F_m) at dusk. The maximum excitation pressure over PS II (Q_m) was calculated as $Q_m = 1 - [(\Delta F/F_m')/(F_v/F_m)]$ (Iglesias-Prieto et al. 2004). $\Delta F/F_m'$ was also recorded *in situ* on coral colonies of *O. annularis* ($n = 38$) and *O. franksi* ($n = 67$) randomly distributed over the full depth range of each species. In order to calculate Q_m on these colonies, we estimated F_v/F_m based on a linear regression with data obtained from a sub-sample of colonies randomly distributed over the same depth range ($n = 10$ and $n = 21$ for *O. annularis* and *O. franksi*, respectively). Pearson's correlation coefficients revealed a strong positive correlation between F_v/F_m and depth in both *O. annularis* and *O. franksi* ($R^2 = 0.85$, $p < 0.01$ and $R^2 = 0.83$, $p < 0.01$), indicating a reliable prediction of F_v/F_m across depths. We used linear regression models to explore the relationship between Q_m and depth for *O. annularis* and *O. franksi* based on evidence that Q_m varies in a pattern that is roughly linear with depth in other coral species (Iglesias-Prieto et al. 2004). An Analysis of Covariance (ANCOVA) was conducted to test for differences in slopes and intercepts among regression models (interaction of species with depth). Due to technical issues with the Diving-PAM (loss in hermeticity), samples from the transplant experiment were transported from the transplant sites to the boat in a dark container to record measurements. During this short period of dark acclimation (<5 min), some components of the non-photochemical quenching could have relaxed (Ralph and Gademann 2005), leading to a slight, yet nearly constant, underestimation of the $\Delta F/F_m'$ recorded at noon and, as a result, of Q_m in all corals. Analyses were conducted using R version 3.6.1 (R Core Team 2015).

Microbiome

Small Subunit Ribosomal RNA (16S) amplicon library preparation and sequencing, sequence quality control and initial data processing

We quantified coral-associated microbiome communities in coral transplants to test if adaptive divergence between *O. annularis* and *O. franksi* is in part due to their microbial communities. Tissue

150 samples were collected at the end of the transplant experiment using 1/8" metal corers by divers
151 wearing Nitrile gloves and were immediately deposited in whirl pack bags. Once returned to the boat,
152 each sample was gently washed with filter-sterile (0.2 µm) seawater, deposited in a sterile cryovial, and
153 immediately preserved in liquid nitrogen. We extracted DNA from coral tissue samples using the MoBio
154 Powersoil DNA Isolation Kit (MoBio Laboratories). Two-stage amplicon PCR was performed on the V4
155 region of the 16S small subunit prokaryotic rRNA gene (Apprill et al. 2015; Roitman et al. 2020). First, 30
156 PCR cycles were performed using 515F and 806R primers (underlined) with linker sequences at the 5'
157 ends: 515F_link (5'-ACA CTG ACG ACA TGG TTC TAC AGT GCC AGC MGC CGC GGT AA-3') and 806Rb_link
158 (5'-TAC GGT AGC AGA GAC TTG GTC TGG ACT ACH VGG GTW TCT AAT-3'). Each 20 µL PCR reaction was
159 prepared with 9 µL 5Prime HotMaster Mix (VWR International), 1 µL forward primer (10 µM), 1 µL
160 reverse primer (10 µM), 1 µL template DNA (~20 ng/ µL), and 8 µL PCR-grade water. PCR amplifications
161 consisted of a 3 min denaturation at 94 °C; 30 cycles of 45 s at 94 °C, 60 s at 50 °C and 90 s at 72 °C; and
162 10 min at 72 °C. Amplicons were barcoded with Fluidigm barcoded Illumina primers (8 cycles) and
163 pooled in equal concentrations for sequencing. The amplicon pool was purified with AMPure XP beads
164 and sequenced on the Illumina MiSeq sequencing platform at the DNA Services Facility at the University
165 of Illinois at Chicago. Sequences were submitted to the National Center for Biotechnology Information
166 (NCBI) Short Read Archive (SRA) under project number PRJNA717688.

167 Initial processing of 16S libraries was performed using the Quantitative Insights Into Microbial Ecology
168 (QIIME; v1.9) package (Caporaso et al. 2010b). Primer sequences were trimmed, paired-end reads
169 merged, and QIIME's default quality-control parameters were used to split libraries among samples.
170 Chimeras were removed and 97%-similarity OTUs picked using USEARCH 7.0 (Edgar 2010), QIIME's
171 subsampled open-reference OTU-picking protocol (Rideout et al. 2014), and the 97% GreenGenes 13_8
172 reference database (McDonald et al. 2012). Taxonomy was assigned using UCLUST and reads were
173 aligned against the GreenGenes database using PyNAST (Caporaso et al. 2010a). FastTreeMP (Price et al.
174 2010) was used to create a bacterial phylogeny with constraints defined by the GreenGenes reference
175 phylogeny. OTUs classified as "unknown" (*i.e.*, sequences not classified at the kingdom level),
176 chloroplast, mitochondria, or other potential contaminants were removed. Low coverage samples (< 223
177 useable reads) were omitted. Unless otherwise stated, downstream microbiome analyses and figure
178 generation were performed in R version 3.2.5 (R Core Team 2015) using the phyloseq and ggplot2
179 packages (Wickham 2009; McMurdie and Holmes 2013).

180

ß-diversity group significance and differential abundance testing

To quantify differences among treatments, we used weighted UniFrac (wUniFrac) dissimilarity matrices using OTU-level relative abundances. Significant differences in bacterial assemblages were assessed by permutational multivariate analysis of variance (PERMANOVA) with wUniFrac distances and the explanatory variables host species and depth (*i.e.*, vegan::adonis) (Oksanen et al. 2017). Both overall (*i.e.*, *O. annularis* and *O. franksi*) and species-specific models (*i.e.*, *O. annular*is or *O. franksi*) were tested. Heatmaps of OTU abundances were created using the phyloseq::plot_heatmap function (McMurdie and Holmes 2013). Within-category microbiome variability (*i.e.*, wUniFrac distance) was calculated in QIIME using the make_distance_boxplots function, which also assesses significant differences in microbiome variability among categories via pairwise, nonparametric t-tests (1000 Monte Carlo permutations) with Bonferroni correction. To test for significant differences in OTU abundances across host species and depths, we employed negative binomial modelling using DESeq2 (McMurdie and Holmes 2013; Love et al. 2014). Both the overall (*i.e.*, *O. annularis* and *O. franksi*) and species-specific models (*i.e.*, *O. annular*is or *O. franksi*) were tested. P-values for the significance of contrasts were generated based on Wald statistics, and false discovery rates were calculated using the Benjamini–Hochberg procedure.

Microalgal communities

Internal Transcribed Spacer 2 rRNA (ITS2) amplicon library preparation, sequencing, and initial processing

To quantify differences in dinoflagellate communities across species and depths, we used a two-stage amplicon PCR on the same DNA that was extracted and used for the 16S amplification. We amplified the Internal Transcribed Spacer 2 (ITS2) rRNA marker gene commonly used for identification of Symbiodiniaceae (Hume et al. 2019). The primers used to quantify differences in the symbiotic algal communities were modified versions of the ITS-DINO forward (5'-ACA CTG ACG ACA TGG TTC TAC AGT GAA TTG CAG AAC TCC GTG-3') and ITS2Rev2 (5'- TAC GGT AGC AGA GAC TTG GTC TCC TCC GCT TAC TTA TAT GCT T-3') (Stat et al. 2009) that include the universal primer sequences required for Illumina MiSeq amplicon sequencing, namely common sequence 1 (CS1) and common sequence 2 (CS2). The PCR amplification was structured as follows: 2 min of denaturation at 94 °C; 35 cycles of 45 s at 94 °C, 60 s at 55 °C, and 90 s at 68 °C; then finally 7 min at 68 °C. Once the PCR reactions were finished, samples were held at 4 °C before sequencing. Samples were sequenced using the Illumina MiniSeq platform at the DNA Services Facility at the University of Chicago, Illinois. Sequences were submitted to SymPortal for

processing and quality checks (Hume et al. 2019). Quality checking was performed using mothur (Schloss et al. 2009), followed by taxonomic identification using blastn. The SymPortal pipeline then subdivides sequences into genus groupings and identified type profiles, referred to as defining intragenomic sequence variants (DIVs). Type profiles were only identified if a variant contained more than 200 sequences, and the sequences were subsequently named based on whether they had been used in the definition of the DIVs. The resulting absolute and relative count tables were imported into R version 3.5.2 (R Core Team 2015) for downstream analyses and figure generation using the phyloseq (McMurdie and Holmes 2013), vegan (Oksanen et al. 2017), microbiome (Lahti and Shetty 2017), and ggplot2 (Wickham 2009) packages.

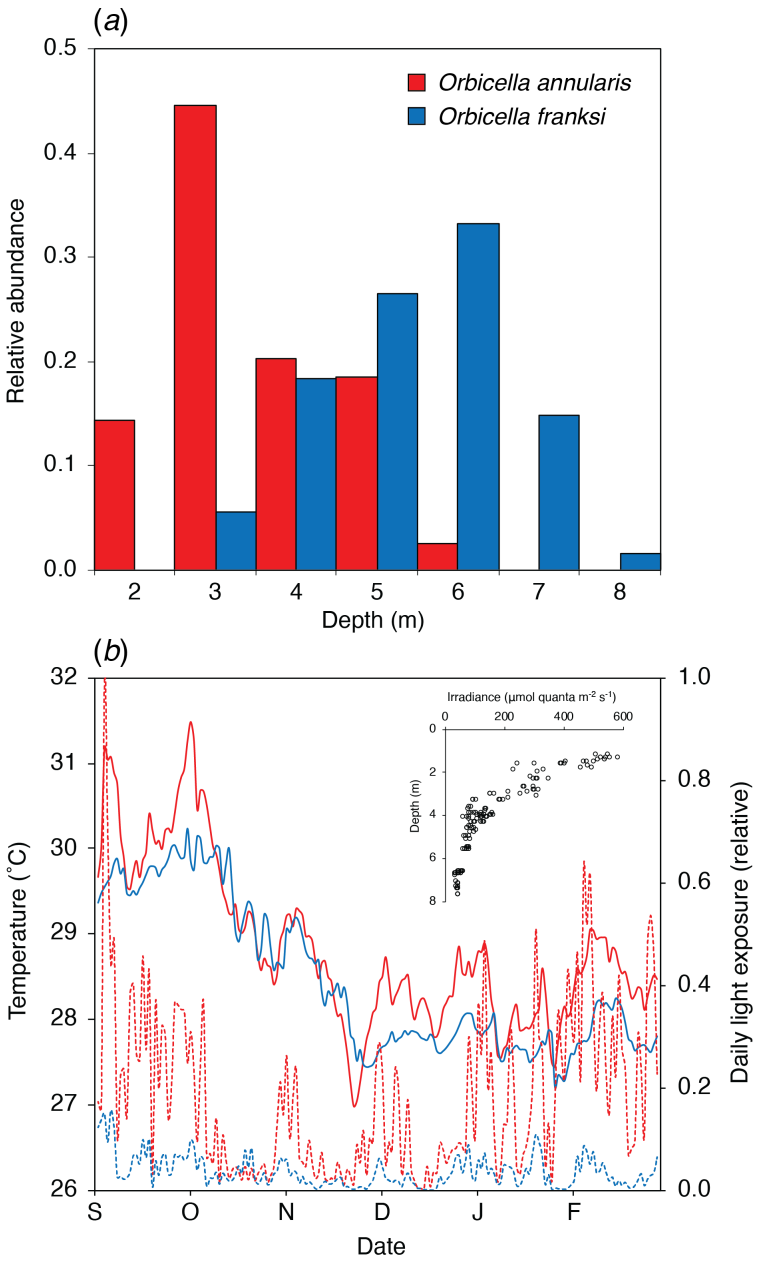
ß-diversity group significance testing

To compare dinoflagellate communities across samples, we constructed Bray-Curtis and Jaccard dissimilarity matrices using absolute abundances. Significant differences in bacterial communities between sample types were assessed by PERMANOVA with Bray-Curtis and Jaccard distances and explanatory variables including host species, season, and depth using the adonis function from the vegan package (Oksanen et al. 2017). We tested overall models that encompassed both species as well as species-specific models.

RESULTS

Temperature and irradiance are higher and more variable in shallow environments

The K_d near the transplant sites was 0.40 m^{-1}, indicating that corals from the shallow (3.5 m) and deep (9.5 m) sites receive respectively ~25% and ~2% sea surface irradiance. Across the vertical distribution range of each species (**Fig 1a**), it is estimated that the light intensity varies between 18% and 62% sea surface irradiance for *O. annularis* and between 5% and 33% for *O. franksi*. Relative light levels recorded by data loggers indicated that the light exposure was nearly 5 times more variable in shallow water than in deep water. Daily temperatures were significantly higher in the shallow site (28.85 $\pm$ 0.96 ^{o}C, mean $\pm$ s.d.) than in the deep site (28.46 $\pm$ 0.88 ^{o}C; t-value = 3.92, $p < 0.001$; **Fig. 1b**). However, based on the scaling quotient of temperature (Q_{10}) of *Orbicella* spp. (Scheufen et al. 2017), it is estimated that the metabolic rate variation due to differences in temperature among sites is negligible (~5%).



238

239 ***Figure 1.*** (*a*) Vertical distribution of *O. annularis* and *O. franksi* around the transplant sites in Bocas del
240 Toro, Panamá, previously established as part of the long-term monitoring of coral spawning (Levitan et
241 al. 2011). (*b*) Variation of the mean daily temperature (continuous lines) and relative light exposure
242 (discontinuous lines) at the shallow (red) and deep (blue) transplant sites. The inset shows the light
243 intensity variation across depths used to calculate the local K_d.
244

245

246

O. annularis experiences greater mortality in deep environments

Transplantation of *O. annularis* S-D (Δ_{depth} = 6 m) resulted in 26% mortality (Fisher exact test: p = 0.003) and was significantly higher than that of *O. franksi* colonies transplanted D-D (4% mortality, Fisher exact test: p = 0.04). *O. franksi* therefore has an advantage of 26% over *O. annularis* in deep habitats. In contrast, *O. franksi* when transplanted D-S did remarkably well with only 2% mortality (Fisher exact test: p = 0.63). Mortality of the two species was not significantly different (0% mortality, Fisher exact test: p = 0.60), suggesting that *O. franksi* in shallow areas has no perceivable short-term (< six months) disadvantage relative to *O. annularis* (Fisher exact test: p = 0.60).

Photoacclimation of O. annularis is insufficient to compensate for reduced light

Symbionts of *O. annularis* exhibited a significant increase in F_v/F_m when transplanted S-D (0.622 ± 0.034) relative to corals transplanted S-S (0.541 ± 0.007) (t-value = -6.25, p < 0.01). On the contrary, symbionts of *O. franksi* transplanted D-S experienced a reduction in F_v/F_m (0.470 ± 0.052) relative to D-D transplants (0.630 ± 0.020; t-value = 0.55, p < 0.01). Transplantation of *O. annularis* S-D induced a significant reduction in Q_m (0.008 ± 0.076), relative to S-S transplantation (0.216 ± 0.163; t-value = 3.67, p < 0.01) (**Fig. 2*a*)**; while *O. franksi* exhibited a significant increase in Q_m (0.226 ± 0.156) when transplanted D-S, relative to D-D transplants (0.073 ± 0.056; t-value = 3.26, p < 0.01) (**Fig. 2*b***).

Estimations of Q_m on coral colonies along the vertical distribution of each species ranged from 0.099 to 0.673 in *O. annularis* and from 0.020 to 0.492 in *O. franksi* (**Fig. 2*c***). We found a significant species by depth interaction ($F_{(1,102)}$=28.78, p<0.001), indicating that the slope of the regression model describing the relationship between Q_m and depth was significantly different between species, being more than twice as pronounced in *O. annularis* (m=-0.13; R^2=0.71, p<0.001) than in *O. franksi* (m=-0.05; R^2=0.50, p<0.001). The linear regression of Q_m with depth indicated that the potential depth limit described by the bioenergetics of the coral-algae symbiosis (*i.e.*, where Q_m reaches the minimum theoretical value of 0) is 5.5. m for *O. annularis* and 7.8 m for *O. franksi* (**Fig. 2c**), which nearly coincide with the observed lower limit of distribution of both species in the study area (**Fig. 1a**).

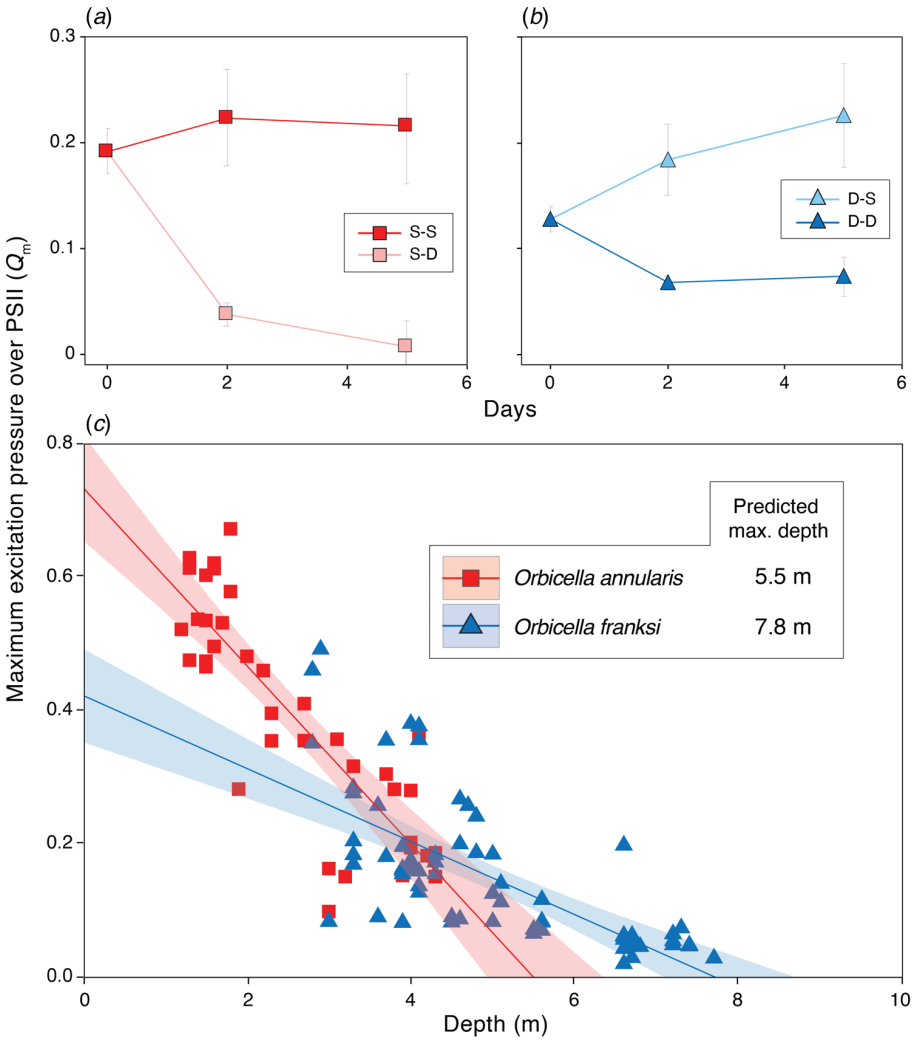
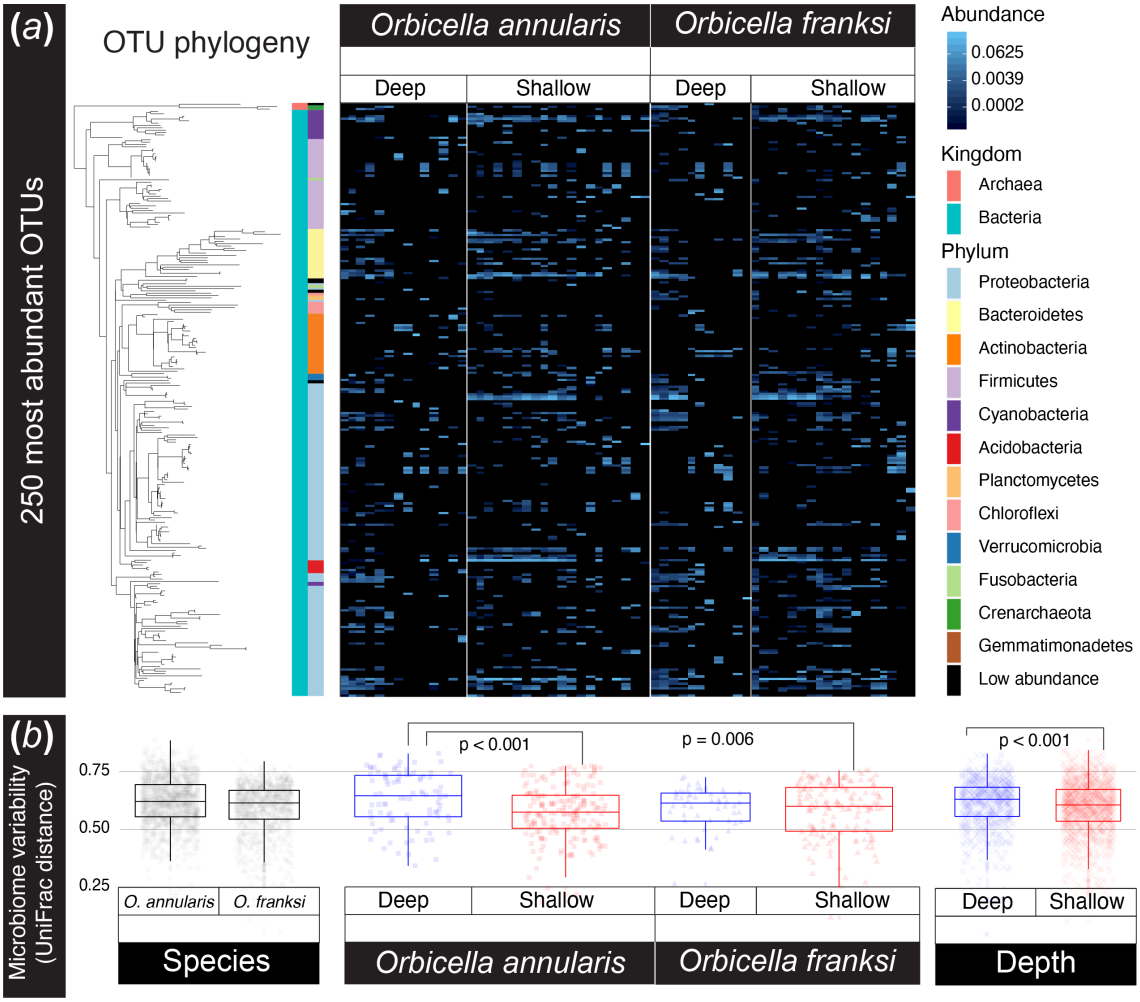


Figure 2. Photoacclimation responses of *Orbicella* spp. across depths. Maximum excitation pressure over PS II (Q_m) is shown pre- and post-transplantation for *O. annularis* (*a*) and *O. franksi* (*b*). Values obtained in *O. annularis* transplanted S-S are shown in dark red while those transplanted S-D in pink. Values from *O. franksi* transplanted D-D are shown in dark blue while those transplanted D-S in light blue. (*c*) Q_m variation in *O. annularis* (red) and *O. franksi* (blue) along a depth gradient. A linear model was used to fit the data and predict the maximum potential depth limit described by Q_m for *O. annularis* [Q_m = 0.735 – 0.133*depth; R^2=0.71, p<0.001] and *O. franksi* [Q_m = 0.422 - 0.054*depth; R^2=0.50, p<0.001]. Clear lines represent 95% confidence intervals.

Changes in depth produces a major shift in O. annularis microbiome

After quality control, sequencing resulted in a total of 577,930 microbial reads (per sample median: 5,758; per sample mean: 9,173) partitioned across 14,274 unique OTUs. Overall, coral-associated prokaryote communities were significantly structured according to depth (p = 0.001), but not host

286 species (p = 0.12) or depth by species interaction (p = 0.86; PERMANOVA on weighted UniFrac; **Fig. 3**).
287 The change across depths is mainly driven by *O. annularis* (p = 0.01, **Fig. 3**). The strong response of *O.*
288 *annularis* microbiomes to changes in depth can be visualized in differential patterns of OTU abundance
289 among depths (**Fig. 3*a***).



290

291 ***Figure 3.*** *O. annularis* microbiomes vary across timepoints and depths while *O. franksi* communities
292 remain consistent. (*a*) Relative abundances of the 250 most common OTUs reveal distinct patterns
293 among *O. annularis* microbiomes at the two transplant depths while *O. franksi* abundance patterns
294 remain largely consistent across treatments. Each column in the heatmap represents an individual
295 microbiome sample and phylogenetic relationships among OTUs are shown on the left (FastTree
296 maximum-likelihood tree). (*b*) Microbiome variability (*i.e.*, weighted UniFrac distances) was greatest in
297 *O. annularis* corals transplanted to deep waters. Microbiome variability was higher in corals in deep
298 waters than in shallow.
299

Ten bacterial taxa were significantly enriched in shallow-water samples. OTUs enriched in shallow-water coral microbiomes are from the bacterial Orders Acidimicrobiales (1 OTU), Alteromonadales (1), Kiloniellales (2), Lactobacillales (1), Neisseriales (1), Oceanospirillales (3), and Synechococcales (1). The mean $\log_2$ fold change for enriched OTUs was 5.6.

Microbiome variability did not differ significantly between species with *O. annularis* (0.592 ± 0.008; mean UniFrac distance ± standard error) and *O. franksi* fragments (0.582 ± 0.008) ($p_{adj} = 0.358$). In contrast, microbiome variability differed significantly between depths, being greatest in *O. annularis* transplanted S-D (0.631 ± 0.013; mean UniFrac distance ± standard error) and significantly higher than *O. annularis* transplanted S-S (0.574 ± 0.008; $p_{adj} < 0.001$) or *O. franksi* transplanted D-S (0.580 ± 0.010; $p_{adj} = 0.006$) (**Fig. 3*b***). The larger microbiome variability in *O. annularis* transplanted deep is consistent with higher mortality and limited photoacclimation potential.

Symbiodiniaceae communities vary across species

Algal communities of *O. annularis* were significantly different from those of *O. franksi* regardless of the depth to which they were transplanted to ($p < 0.05$; pairwise PERMANOVA on a Bray-Curtis matrix). Symbiodiniaceae genotypes belonging to the genus *Symbiodinium* (ITS2 type A3) and *Cladocopium* (C3an, C3an/C3, C7, and C7f) occurred in both coral species, although *Cladocopium* genotypes were more abundant in *O. franksi*. Genotypes from the genus *Breviolum* (B1 and B1/B1t) were detected in high abundances in *O. annularis,* and in many colonies from the shallow site (40% of them) were the only dominant symbiont. Only one *O. franksi* colony transplanted D-S hosted a *Breviolum* (B1) population. Genotypes belonging to the genus *Durusdinium* (D1, D1bl, D4, and D4c) were detected only in *O. franksi* transplanted D-D (**Fig. 4**). Neither *O. annularis* nor *O. franksi* Symbiodiniaceae communities were significantly different when transplanted to a different depth ($p > 0.1$; pairwise PERMANOVA on a Bray-Curtis matrix).

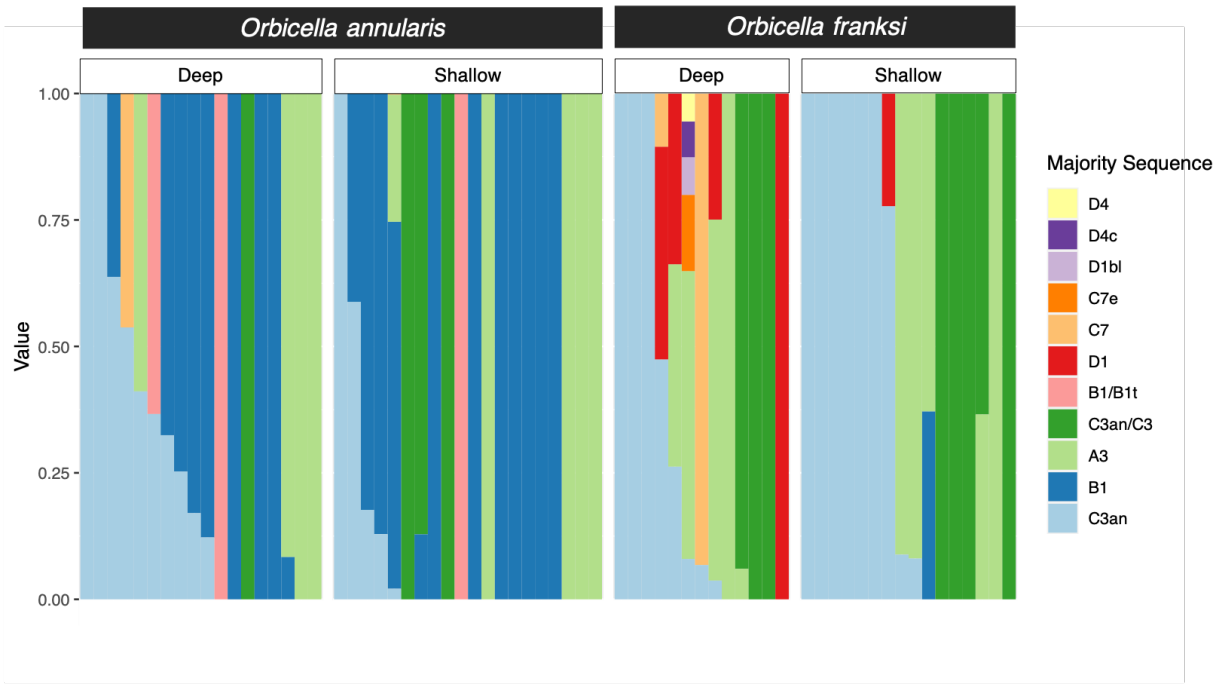


Figure 4. Relative abundance bar plot of Symbiodiniaceae ITS2 profiles identified in *Orbicella* spp. by Symportal (Hume et al. 2019). Variation in Symbiodiniaceae types is shown by species as well as by depth.

DISCUSSION

Our study demonstrates that despite being genetically close (Levitan et al. 2011), *O. annularis* and *O. franksi* have diverged physiologically and occupy distinct light environments in part due to the variation in their associated microbiotas (Symbiodiniaceae and bacterial communities). Following transplantation to deep habitats, *O. annularis* experiences a limited photoacclimation potential and disruption of the photosynthetic performance of its algal symbionts, consistent with increased mortality and significant microbiome community shifts with increased variability. By contrast, *O. franksi* maintained a robust physiological performance, a resilient microbiome composition with no significant community shifts or increased variability, and low mortality at both depths. Our study suggests that *O. annularis* is adapted to shallow environments characterized by a higher and more variable temperature and light regimes, while *O. franksi* is physiologically able to live in both shallow and deep habitats. The niches of these sibling species have diverged, and a large component of the niche separation seems to be related to variations in the photoacclimation capabilities and the microbial community of each species. The absence of *O. franksi* in shallow areas may be related to other ecological aspects such as slow growth in

an area of intense space competition, a restricted morphological plasticity for regulating the light capture (see below) and/or non-random settlement of recruits across depths (*i.e.,* larval habitat choice).

The vertical distribution couples with the photoacclimation capabilities of each species

The vertical distribution of *O. annularis* and *O. franksi* is compressed toward shallower depths in Bocas del Toro compared to other clear-water sites in the Caribbean (*e.g.*, Curaçao (Van Veghel 1994) and Belize (Pandolfi and Budd 2008)). The vertical habitat compression in both species is consistent with the K_d measured in Bocas del Toro (0.40 m^{-1}), which is notably higher than in clear-water sites (0.06 m^{-1} in Curaçao and 0.08 m^{-1} in Belize (Banaszak et al. 1998; Vermeij and Bak 2002)) and reflects the effect of the heavy rainfall patterns and runoff in the region on the optical properties of the water column (Kaufmann and Thompson 2005). This vertical habitat compression is consistent with other coral reefs exposed to water turbidity (Morgan et al. 2020; López-Londoño et al. 2021) and suggest that the light penetration into the water column associated with the local K_d is a determinant factor for the vertical zonation of *Orbicella* spp. Despite local differences in the vertical distribution ranges, *O. annularis* consistently occupies well-lit shallow areas of reefs where the potential for increased photosynthesis and calcification rates drives a steep competition for space with other corals. In contrast, *O. franksi* consistently dominates deeper reef areas characterized by low-light conditions and reduced coral-growth rates (Cohen and Dubinsky 2015).

Our findings indicate that *O. annularis* experiences an almost complete loss of photosynthetic activity when transplanted deep. *O. annularis* fragments photoacclimate to low-light conditions by increasing the light energy conversion efficiency (*i.e*, increase in F_v/F_m) (Hoegh-Guldberg and Jones 1999; Gorbunov et al. 2001). However, the extremely low values of Q_m reflect a trivial photosynthetic contribution of *O. annularis* symbionts to the host metabolism due to light-limited photosynthesis (Iglesias-Prieto et al. 2004), suggesting that the photoacclimation potential is insufficient to compensate for the low-light conditions of deep environments. Photoacclimation of *O. franksi* fragments transplanted to the shallow environment resulted in an increased fraction of photo-inactivated PSII reaction centers and capacity for thermal dissipation of excessive light energy absorbed (Hoegh-Guldberg and Jones 1999; Gorbunov et al. 2001). But in contrast to *O. annularis*, the estimated Q_m in *O. franksi* do not indicate the occurrence of chronic photoinhibition in the shallow environment nor light-limitation in the deep environment, suggesting that *O. franksi* can maintain a more robust physiological performance across depths. The photoacclimation responses of both species in the transplant experiment were consistent with the rates

of change in Q_m across their vertical distribution range, which collectively suggest that the symbiotic algae of *O. annularis* are more sensitive to changes in light intensity with depth than symbionts of *O. franksi*.

Colony morphology can help modulate the light capture and photosynthetic energy acquisition along the vertical distribution range of corals (Hoogenboom et al. 2008; Kaniewska et al. 2011). The dominance of *O. annularis* in shallow habitats correlates with its faster vertical growth among *Orbicella* species (Weil and Knowlton 1994). Its morphology (typically columnar) helps regulate the distribution of light energy for symbiotic algae across the colony surface, representing an advantageous strategy in high-light environments because it reduces the coral tissue area subjected to excessive irradiance (Kaniewska et al. 2011). When transplanted deep, this morphology may lead to acute light energy limitation which, in combination with the insufficient acclimation potential to compensate for low-light, can lead to negative energetic balances for the whole colony and eventual death. *O. franksi,* on the other hand, produce plate-like colonies to maximize light capture in deep environments. When transplanted to shallow well-lit environments, despite a potential for successful photoacclimation as indicated by our results, the plate-like morphology limits the capacity to regulate the internal light climate and allows very slow vertical growth. This slow growth makes *O. franksi* a poor competitor, likely explaining why this species is rare in shallow areas. Alternatively, and not mutually exclusive, their larvae may preferentially settle in low light environments. In fact, adaptation and strong selection across depths, may promote the evolution of habitat choice.

Host species drive symbiont communities

Species-specific associations with algal symbionts with contrasting photoacclimation capabilities may be a key axis of differentiation between *O. annularis* and *O. franksi.* Despite the higher and more variable temperature and light intensity in shallow areas, which are known conditions that promote the association with *Durusdinium trenchii* in other corals (LaJeunesse et al. 2009), this dinoflagellate was not detected in *O. annularis* colonies. Surprisingly, this thermotolerant symbiont (ITS type D1/D1bl) was found in nearly 20% of *O. franksi* colonies from the deep environment. The increased abundance of *D. trenchii* in *O. franksi* may be related with the runoff impacts in the water column (*e.g.*, sedimentation and nutrient enrichment), a reduction in light penetration, and the mechanisms by which the coral-algae symbiosis interact with these environmental conditions (Garren et al. 2006). The prevalence of *Breviolum* genotypes in *O. annularis* and *Cladocopium* genotypes in *O. franksi,* both in the shallow and

deep transplant sites, is consistent with previous reports (LaJeunesse 2002; Garren et al. 2006) and may indicate the formation of stable associations explained by the photoacclimative capabilities of dinoflagellates and the variability of physical factors within the vertical distribution range of each coral species (LaJeunesse 2002; Iglesias-Prieto et al. 2004). The ITS2 analysis has a low resolution to differentiate linages within the same genus in symbiotic algal communities (LaJeunesse and Thornhill 2011; Stat et al. 2011). It is possible that complementary analysis with other molecular markers improves the phylogenetic resolution of Symbiodiniaceae (*i.e.,* species or population level), detecting differences in cryptic species/populations of *Cladocopium* spp. or *Breviolum* spp. uniquely associated with each *Orbicella* species like in other depth-segregated anthozoans (Prada et al. 2014; Pochon et al. 2015).

Microbiome communities vary across depths and are enriched in shallow habitats

Several Endozoicomonas OTUs were significantly enriched in shallow habitats. Endozoicimonaceae are diverse gammaproteobacterial symbionts of numerous marine hosts at varying depths and with a wide global distribution (Neave et al. 2016). Members of this group are found in abundance in the tissues of coral species and are considered to be true symbionts of corals which may provide a beneficial function (Bayer et al. 2013; Pantos et al. 2015). Although their function within the coral host is not entirely clear; proposed benefits include nutrient acquisition, microbiome structuring and roles in coral health.

Members of the family Alteromonadaceae and the order Acidimicrobiales were also enriched in shallow areas. Alteromonadaceae belong to a diverse group of heterotrophic gammaproteobacteria known to associate with marine hosts and nutrient rich environments. Members of this group tolerate relatively high temperatures and have been used in coral probiotic studies as coral-associated bacteria capable of scavenging free radicals (Dungan et al. 2020), and therefore could provide similar benefits in shallow, high-light environments. Similarly, Acidimicrobiales are known to be planktonic free-living photo-heterotrophs found in both temporal and tropical photic zones (Angly et al. 2016) and are associated with DOM in marine environments (Osterholz et al. 2018).

Finally, corals in shallow areas were also enriched for *Alloiococcus* and *Synechococcus*. *Alloiococcus* belongs to the group of gram-positive lactic acid bacteria, which are recognized for producing bacterial growth inhibitors that function to deter invading bacteria in their hosts (Ringø et al. 2018). *Synechococcus* is a photoautotrophic cyanobacterium found in surface waters harbouring abundant light. Both corals and their symbiotic algae are known to actively feed on *Synechococcus* (Jeong et al.

2012; McNally et al. 2017) which is often found as a member of the coral surface mucus microbiome (Marchioro et al. 2020). As a food for corals, it has been suggested that nitrogen-rich *Synechococcus* cells may increase bleaching recovery and coral health (Meunier et al. 2019).

There is a continuing debate as to the relative role of coral host vs. environment in shaping coral microbiomes. This study demonstrates that the responsiveness of coral microbiomes to environmental conditions differs significantly even among very closely related coral species. These differences in microbiome shifts may be related to the resilience of the coral host and its associated algal community to a particular habitat. Pantos et al. (Pantos et al. 2015) found that environment is the major driver of microbiome structure in *Seriatopora hysterix*, not host genotype or Symbiodiniaceae strain. Our results do not contradict this finding but suggest that responsiveness to environmental conditions can differ significantly even among very closely related coral taxa.

Implications for coral reef conservation

A key finding in our study with implications for coral restoration is the increased mortality of *O. annularis* when transplanted to low-light environments. We suggest that to enhance survivorship during restoration, the particular light environment of source populations should be similar to the transplant sites. In this study, due to the high vertical attenuation of light (K_d = 0.40 m^{-1}), a 6 m increase in depth resulted in an order of magnitude reduction in irradiance and increased mortality of *O. annularis* by 26%. In a clear-water site (*e.g.*, K_d = 0.06 m^{-1}), this response would be expected to occur with an increase in depth of ~40 m. Giving the expensive nature of coral restoration, equating the light environment of donor and transplant sites will likely increase yield and decrease costs. Minimally, our approach can be used to estimate the maximum theoretical depth for each species in a given location with certain water optical quality, thereby providing guidance when choosing the location and depth for coral transplantation.

The second aspect of our findings is related to microbiome composition in different habitats across reefs. Shallow water reefs are areas of high stress with strong variations in light, temperature and salinity, strong changes in water motion and sediment transport, and more ecological variability. Our study suggests that the microbiome of shallow water specialists like *O. annularis* is fine-tuned to this environment and a reduction in the light field can cascade into drastic changes to host-associated microbial community composition. Increases in temperature as a result of climate change has affected primarily shallow water corals (Bridge et al. 2013; Hughes et al. 2018), suggesting that instability in the

462 coral microbiomes of shallow-water corals will increase, likely accelerating coral decline of these reef
463 areas.

464 Lastly, subtle differences in the water optical conditions can result in changes in the underwater light
465 environment and the vertical distribution of coral species. Most coral reefs around the globe are
466 currently threatened by the direct effects of sediments, pollutants and nutrients associated with coastal
467 development and terrestrial runoff (Carlson et al. 2019). These conditions affect the water optical
468 quality and, as a consequence, the light climate of corals and the survivorship of species at different
469 depths. Although previous studies have suggested that deep-water species are more sensitive to
470 changes in water optical conditions (Vermeij and Bak 2002), our results suggest that at least some
471 shallow-water specialists, like *O. annularis*, can be extremely vulnerable to these changes as their
472 physiology/morphology is specialized for high light habitats. As the degradation of water optical
473 properties in coral reefs continue, shallow-water specialists, which are typically major reef-building
474 species, will likely become rare, shifting the structural and functional integrity of reefs.

475 CONCLUSION

476 Our study suggests that the sibling coral species, *O. annularis* and *O. franksi*, are adapted to distinctive
477 light environments along depth gradients. The limited photoacclimation potential and less robust
478 microbiome community restricts *O. annularis* to shallow, high-light environments. *O. franksi* is more
479 versatile, but other ecological aspects such as slow growth in areas of intense space competition
480 restricts the species to deep environments. These contrasting responses associated with the microbial
481 communities highlight the importance of niche specialization in symbiotic corals for the maintenance of
482 species diversity. Our study has implications on coral reef restoration efforts, providing guidance when
483 choosing the location, depth and light environment for coral transplantation.

484 ACKNOWLEDGMENTS

485 Arcadio Castillo Díaz, Gabriel Jácome and Plinio Góndola from the Smithsonian Tropical Research
486 Institute assisted with field operations at the Bocas del Toro field station. Gaby Swain helped on sample
487 processing. Dr. Benjamin Hume provided assistance on Symbiodiniaceae analysis through the SymPortal
488 framework.

FUNDING STATEMENT

This work was supported by NSF grants OCE 1442206 and OCE 1642311; Pennsylvania State University startup funds to MM and RI-P; and NOAA grant NA19NOS4820132. CP was funded by grants from NSF (OIA) 2032919 and USDA National Institute of Food and Agriculture (Hatch) 1017848.

COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

Angly FE, Heath C, Morgan TC, Tonin H, Rich V, Schaffelke B, Bourne DG, Tyson GW (2016) Marine microbial communities of the Great Barrier Reef lagoon are influenced by riverine floodwaters and seasonal weather events. Peerj 4:e1511

Apprill A, McNally S, Parsons R, Webe L (2015) Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. Aquat Microb Ecol 75:129–137

Banaszak A, Lesser M, Kuffner I, Ondrusek M (1998) Relationship between ultraviolet (UV) radiation and mycosporine-like amino acids (MAAS) in marine organisms. Bull Mar Sci 63:617-628

Bayer T, Neave MJ, Alsheikh-Hussain A, Aranda M, Yum LK, Mincer T, Hughen K, Apprill A, Voolstra CR (2013) The microbiome of the Red Sea coral Stylophora pistillata is dominated by tissue-associated Endozoicomonas bacteria. Appl Environ Microbiol 79:4759-4762

Bridge TC, Hoey AS, Campbell SJ, Muttaqin E, Rudi E, Fadli N, Baird AH (2013) Depth-dependent mortality of reef corals following a severe bleaching event: implications for thermal refuges and population recovery. F1000Research 2

Caporaso JG, Bittinger K, Bushman FD, DeSantis TZ, Andersen GL, Knight R (2010a) PyNAST: a flexible tool for aligning sequences to a template alignment. Bioinformatics 26:266-267

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JI (2010b) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7:335-336

Carlson RR, Foo SA, Asner GP (2019) Land use impacts on coral reef health: a ridge-to-reef perspective. Front Mar Sci 6:562

Cohen I, Dubinsky Z (2015) Long term photoacclimation responses of the coral Stylophora pistillata to reciprocal deep to shallow transplantation: Photosynthesis and calcification. Front Mar Sci 2

Dungan AM, Bulach D, Lin H, van Oppen MJH, Blackall LL (2020) Development of a free radical scavenging probiotic to mitigate coral bleaching. bioRxiv:2020.2007.2002.185645

Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26:2460-2461

Fukami H, Budd AF, Levitan DR, Jara J, Kersanach R, Knowlton N (2004) Geographical differences in species boundaries among members of the *Montastraea annularis* complex based on molecular and morphological markers. Evolution 58:324-337

Garren M, Walsh SM, Caccone A, Knowlton N (2006) Patterns of association between Symbiodinium and members of the Montastraea annularis species complex on spatial scales ranging from within colonies to between geographic regions. Coral Reefs 25:503-512

Gilbert SF, McDonald E, Boyle N, Buttino N, Gyi L, Mai M, Prakash N, Robinson J (2010) Symbiosis as a source of selectable epigenetic variation: taking the heat for the big guy. Phil Trans R Soc B 365:671-678

Gorbunov MY, Kolber ZS, Lesser MP, Falkowski PG (2001) Photosynthesis and photoprotection in symbiotic corals. Limnol Oceanogr 46:75-85

Hereford J (2009) A quantitative survey of local adaptation and fitness trade-offs. Am Nat 173:579-588

Hoegh-Guldberg O, Jones RJ (1999) Photoinhibition and photoprotection in symbiotic dinoflagellates from reef-building corals. Mar Ecol Prog Ser 183:73-86

Hoogenboom MO, Connolly SR, Anthony KRN (2008) Interactions between morphological and physiological plasticity optimize energy acquisition in corals. Ecology 89:1144-1154

Hughes TP, Kerry JT, Baird AH, Connolly SR, Dietzel A, Eakin CM, Heron SF, Hoey AS, Hoogenboom MO, Liu G, McWilliam MJ, Pears RJ, Pratchett MS, Skirving WJ, Stella JS, Torda G (2018) Global warming transforms coral reef assemblages. Nature 556:492-496

Hume BCC, Smith EG, Ziegler M, Warrington HJM, Burt JA, LaJeunesse TC, Wiedenmann J, Voolstra CR (2019) SymPortal: a novel analytical framework and platform for coral algal symbiont next-generation sequencing ITS2 profiling. Mol Ecol Resour 19:1063-1080

Iglesias-Prieto R, Beltran VH, LaJeunesse TC, Reyes-Bonilla H, Thome PE (2004) Different algal symbionts explain the vertical distribution of dominant reef corals in the eastern Pacific. Proc R Soc Lond B 271:1757-1763

Jeong HJ, Du Yoo Y, Kang NS, Lim AS, Seong KA, Lee SY, Lee MJ, Lee KH, Kim HS, Shin W (2012) Heterotrophic feeding as a newly identified survival strategy of the dinoflagellate Symbiodinium. Proc Natl Acad Sci USA 109:12604-12609

Kaniewska P, Magnusson SH, Anthony KRN, Reef R, Kühl M, Hoegh-Guldberg O (2011) Importance of macro- versus microstructure in modulating light levels inside coral colonies. J Phycol 47:846-860

Kaufmann KW, Thompson RC (2005) Water temperature variation and the meteorological and hydrographic environment of Bocas del Toro, Panama. Caribb J Sci 41:392-413

Kellogg CA, Piceno YM, Tom LM, DeSantis TZ, Gray MA, Zawada DG, Andersen GL (2013) Comparing bacterial community composition between healthy and white plague-like disease states in Orbicella annularis using PhyloChip™ G3 microarrays. PLos ONE 8:e79801

Kemp DW, Thornhill DJ, Rotjan RD, Iglesias-Prieto R, Fitt WK, Schmidt GW (2015) Spatially distinct and regionally endemic Symbiodinium assemblages in the threatened Caribbean reef-building coral Orbicella faveolata. Coral Reefs 34:535-547

Kirk JTO (2011) Light and photosynthesis in aquatic ecosystems. Cambridge University Press, New York

Lahti L, Shetty S (2017) microbiome R package. Tools for microbiome analysis in R.

LaJeunesse T (2002) Diversity and community structure of symbiotic dinoflagellates from Caribbean coral reefs. Mar Biol 141:387-400

LaJeunesse TC, Thornhill DJ (2011) Improved resolution of reef-coral endosymbiont (Symbiodinium) species diversity, ecology, and evolution through psbA non-coding region genotyping. PLos ONE 6:e29013

LaJeunesse TC, Smith RT, Finney J, Oxenford H (2009) Outbreak and persistence of opportunistic symbiotic dinoflagellates during the 2005 Caribbean mass coral bleaching event. Proc R Soc B 276:4139-4148

Levitan D, Fogarty N, Jara J, Lotterhos K, Knowlton N (2011) Genetic, spatial, and temporal components to precise spawning synchrony in reef building corals of the *Montastraea annularis* species complex. Evolution 65:1254–1270

López-Londoño T, Galindo-Martínez CT, Gómez-Campo K, González-Guerrero LA, Roitman S, Pollock FJ, Pizarro V, López-Victoria M, Medina M, Iglesias-Prieto R (2021) Physiological and ecological consequences of the water optical properties degradation on reef corals. Coral Reefs 40:1243-1256

Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 15:550

Marchioro GM, Glasl B, Engelen AH, Serrão EA, Bourne DG, Webster NS, Frade PR (2020) Microbiome dynamics in the tissue and mucus of acroporid corals differ in relation to host and environmental parameters. Peerj 8:e9644

McDonald D, Price MN, Goodrich J, Nawrocki EP, DeSantis TZ, Probst A, Andersen GL, Knight R, Hugenholtz P (2012) An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. ISME J 6:610-618

McMurdie PJ, Holmes S (2013) phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLos ONE 8:e61217

McNally SP, Parsons RJ, Santoro AE, Apprill A (2017) Multifaceted impacts of the stony coral Porites astreoides on picoplankton abundance and community composition. Limnol Oceanogr 62:217-234

Meunier V, Bonnet S, Pernice M, Benavides M, Lorrain A, Grosso O, Lambert C, Houlbrèque F (2019) Bleaching forces coral's heterotrophy on diazotrophs and Synechococcus. ISME J 13:2882-2886

585 Morgan KM, Moynihan MA, Sanwlani N, Switzer AD (2020) Light Limitation and Depth-Variable Sedimentation
586 Drives Vertical Reef Compression on Turbid Coral Reefs. Frontiers in Marine Science 7:931
587 Neave MJ, Apprill A, Ferrier-Pagès C, Voolstra CR (2016) Diversity and function of prevalent symbiotic marine
588 bacteria in the genus Endozoicomonas. Appl Microbiol Biotechnol 100:8315-8324
589 Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos
590 P, Stevens MHH, Szoecs E, Wagner H (2017) vegan: Community Ecology Package
591 Osterholz H, Kirchman DL, Niggemann J, Dittmar T (2018) Diversity of bacterial communities and dissolved organic
592 matter in a temperate estuary. FEMS Microbiol Ecol 94:fiy119
593 Pandolfi JM, Budd AF (2008) Morphology and ecological zonation of Caribbean reef corals: the Montastraea
594 'annularis' species complex. Mar Ecol Prog Ser 369:89-102
595 Pandolfi JM, Lovelock CE, Budd AF (2002) Character release following extinction in a Caribbean reef coral species
596 complex. Evolution 56:479-501
597 Pantos O, Bongaerts P, Dennis PG, Tyson GW, Hoegh-Guldberg O (2015) Habitat-specific environmental conditions
598 primarily control the microbiomes of the coral Seriatopora hystrix. ISME J 9:1916-1927
599 Peixoto RS, Rosado PM, Leite DCdA, Rosado AS, Bourne DG (2017) Beneficial microorganisms for corals (BMC):
600 proposed mechanisms for coral health and resilience. Front Microbiol 8:341
601 Pochon X, Forsman ZH, Spalding HL, Padilla-Gamiño JL, Smith CM, Gates RD (2015) Depth specialization in
602 mesophotic corals (Leptoseris spp.) and associated algal symbionts in Hawai'i. R Soc Open Sci 2:140351
603 Prada C, McIlroy SE, Beltrán DM, Valint DJ, Ford SA, Hellberg ME, Coffroth MA (2014) Cryptic diversity hides host
604 and habitat specialization in a gorgonian-algal symbiosis. Mol Ecol 23:3330-3340
605 Price MN, Dehal PS, Arkin AP (2010) FastTree 2–approximately maximum-likelihood trees for large alignments.
606 PLoS ONE 5:e9490
607 R Core Team (2015) R: A language and environment for statistical computing R Foundation for Statistical
608 Computing. R Foundation for Statistical Computing, Vienna, Austria
609 Ralph P, Gademann R (2005) Rapid Light Curves: a powerful tool to assess photosynthetic activity. Aquat Bot
610 82:222-237
611 Rideout JR, He Y, Navas-Molina JA, Walters WA, Ursell LK, Gibbons SM, Chase J, McDonald D, Gonzalez A, Robbins-
612 Pianka A, others (2014) Subsampled open-reference clustering creates consistent, comprehensive OTU
613 definitions and scales to billions of sequences. Peerj 2:e545
614 Ringø E, Hoseinifar SH, Ghosh K, Doan HV, Beck BR, Song SK (2018) Lactic acid bacteria in finfish—An update. Front
615 microbiol 9:1818
616 Rohwer F, Seguritan V, Azam F, Knowlton N (2002) Diversity and distribution of coral-associated bacteria. Mar Ecol
617 Prog Ser 243:1-10
618 Roitman S, López-Londoño T, Joseph Pollock F, Ritchie KB, Galindo-Martínez CT, Gómez-Campo K, González-
619 Guerrero LA, Pizarro V, López-Victoria M, Iglesias-Prieto R, Medina M (2020) Surviving marginalized reefs:
620 assessing the implications of the microbiome on coral physiology and survivorship. Coral Reefs 39:795-
621 807
622 Rowan R, Knowlton N, Baker A, Jara J (1997) Landscape ecology of algal symbionts creates variation in episodes of
623 coral bleaching. Nature 388:265-269
624 Scheufen T, Kramer WE, Iglesias-Prieto R, Enriquez S (2017) Seasonal variation modulates coral sensibility to heat-
625 stress and explains annual changes in coral productivity. Sci Rep 7:4937
626 Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson
627 CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF (2009) Introducing mothur: open-source,
628 platform-independent, community-supported software for describing and comparing microbial
629 communities. Appl Environ Microbiol 75:7537-7541
630 Stat M, Pochon X, Cowie ROM, Gates RD (2009) Specificity in communities of Symbiodinium in corals from
631 Johnston Atoll. Mar Ecol Prog Ser 386:83-96
632 Stat M, Bird CE, Pochon X, Chasqui L, Chauka LJ, Concepcion GT, Logan D, Takabayashi M, Toonen RJ, Gates RD
633 (2011) Variation in Symbiodinium ITS2 sequence assemblages among coral colonies. PLos ONE 6:e15854
634 Stoddart DR (1969) Ecology and morphology of recent coral reefs. Biol Rev 44:433-498
635 Thompson JR, Rivera HE, Closek CJ, Medina M (2015) Microbes in the coral holobiont: partners through evolution,
636 development, and ecological interactions. Front Cell Infect Microbiol 4:1-20

637 Van Veghel MLJ (1994) Polymorphism in the Caribbean reef building coral Montastrea annularis. Ph.D. thesis.
638 University of Amsterdam, p128
639 Vermeij MJA, Bak RPM (2002) How are coral populations structured by light? Marine light regimes and the
640 distribution of Madracis. Mar Ecol Prog Ser 233:105-116
641 Warner ME, LaJeunesse TC, Robison JD, Thur RM (2006) The ecological distribution and comparative photobiology
642 of symbiotic dinoflagellates from reef corals in Belize: potential implications for coral bleaching. Limnol
643 Oceanogr 51:1887-1897
644 Weil E, Knowlton N (1994) A multi-character analysis of the Caribbean coral Montastraea annularis (Ellis and
645 Solander, 1786) and its two sibling species, M. faveolata (Ellis and Solander, 1786) and M. franksi
646 (Gregory, 1895). Bull Mar Sci 55:151-175
647 Wickham H (2009) ggplot2: elegant graphics for data analysis. Springer, New York

648