REPORT

Colony self-shading facilitates Symbiodiniaceae cohabitation in a South Pacific coral community

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Abstract The ecological success of tropical corals is regulated by symbiotic dinoflagellate algae (Symbiodiniaceae). Corals can associate with multiple Symbiodiniaceae species simultaneously, yet the conditions that permit Symbiodiniaceae cohabitation are not understood. We examined how corals self-shade their own tissues causing within-colony light gradients that drive Symbiodiniaceae photoacclimatory processes and positional genetic disparity. Paired light 'exposed' and 'shaded' samples from 20 coral species were collected from a shallow coral reef (Rarotonga, Cook Islands). Through active chlorophyll fluorometry, rapid light curves revealed that exposed Symbiodiniaceae exhibited 50% higher values in minimum saturating irradiances and demonstrated a shift towards preferential non-photochemical quenching $[1 - Q]$, consistent with higher overall light exposure. High-throughput or targeted DNA sequencing of ITS2 and psbA^{ncr} markers demonstrated that corals harboured distinct and/or differentially abundant Symbiodiniaceae ITS2 sequences (typically rare in relative abundance) or multiple ITS2 intragenomic variant profiles across shaded vs exposed regions. In *Hydnophora* cf. *microconos*, within-colony symbiont genetic disparity was positively correlated with the magnitude of difference in $[1 - Q]$ utilisation. Together, these results suggest that within-colony light gradients produce distinct optical niches that enable symbiont cohabitation via photoadaptation, a phenomenon that is expected to increase the adaptive capacity of corals under future climates.

Introduction

The ecological success of tropical corals is strongly regulated by their mutualistic symbiosis with the Symbiodiniaceae, a family of photosynthetic dinoflagellate algae that live within coral endodermal cells (Muscatine 1990). Tight nutrient recycling between these partners supports the metabolic requirements for coral survival and growth (Muscatine 1990; Gattuso et al. 1999; Davy et al. 2012). The Symbiodiniaceae is a diverse family of 16 primary lineages, with 11 formally described as genera (LaJeunesse et al. 2018; Nitschke et al. 2020; Pochon and LaJeunesse 2021; Yorifuji et al. 2021). Symbiodiniaceae species have evolved different physiological optima for environmental variables (Rowan 2004; Abrego et al. 2008; Howells et al. 2012; Hoadley et al. 2021) and may confer traits (e.g. thermal tolerance) to their host (Berkelmans and Van Oppen 2006; Sampayo et al. 2008; Oliver and Palumbi 2011).

A coral may host multiple Symbiodiniaceae species simultaneously, typically with a single highly abundant 'dominant symbiont' and one or more low abundance

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'background symbionts' (Mieog et al. 2007; Silverstein et al. 2012; Boulotte et al. 2016). Low abundance Symbiodiniaceae may also confer environmental stress resistance to their host by supplying a source of genomic innovation, enhancing the resilience of host–symbiont communities (Bay et al. 2016; Ziegler et al. 2018). Studies have proposed that corals may acclimatise or adapt to warmer oceans through rearrangements of symbiont community compositions favouring thermally tolerant species (Buddemeier and Fautin 1993; Baker et al. 2004; Berkelmans and Van Oppen 2006; Cunning et al. 2018; Claar et al. 2020), a phenomenon observed following temperature-induced coral bleaching (Berkelmans and Van Oppen 2006; LaJeunesse et al. 2009; Cunning et al. 2015; Oladi et al. 2021). However, some tolerant symbiont species are considered less compatible with the nutritional needs of their host (Stat and Gates 2011; Pettay et al. 2015; Matthews et al. 2017; Gabay et al. 2018; Sproles et al. 2020), and perturbed symbiont communities tend to return to their original composition over time if stress is alleviated (Thornhill et al. 2006; Stat et al. 2009b; McGinley et al. 2012). Temporary community shifts suggest a hierarchy to symbiont cohabitation (McIlroy et al. 2019, 2020), however the ecological processes (i.e. competition) governing Symbiodiniaceae species interactions have not been fully resolved, and the heterogeneously distributed resource(s) responsible for within-colony, multiple symbiont species cohabitation has yet to be determined.

Light gradients on coral reefs provide significant opportunity for specialisation of Symbiodiniaceae photobiological traits and species partitioning across particular light environments (Sampayo et al. 2007). Examples of depth-driven light gradients and zonation of Symbiodiniaceae species in depth-generalist coral species are common, leading to hypotheses that Symbiodiniaceae are partitioned into optical niches (Rowan and Knowlton 1995; Sampayo et al. 2007; Frade et al. 2008; Goulet et al. 2019). However, it is unknown if the same notion can be applied broadly to the light microenvironments that exist across individual coral colonies. Corals build morphologically heterogeneous structures that inevitably shelf-shade, producing pronounced light gradients within a single colony (Anthony et al. 2005; Kaniewska et al. 2008; Ow and Todd 2010) which, in turn, may create multiple niches for distinct symbiont species to exploit (Suggett et al. 2017). Spatial diversification of symbionts across individual colonies is evident for several coral species (Rowan and Knowlton 1995; van Oppen et al. 2001; Garren et al. 2006) and certain Symbiodiniaceae genera appear to display consistency regarding their preference for light 'exposed' or 'shaded' coral surfaces (Rowan and Knowlton 1995; Garren et al. 2006; Kemp et al. 2008). Localised or patchy bleaching of individual corals is common (Celliers and Schleyer 2002; Penin et al. 2007), and symbiont-retentive regions may

correlate with the zonation of distinct Symbiodiniaceae species with superior tolerances of high temperatures and irradiance (Rowan et al. 1997; Kemp et al. 2014). Symbionts must make continuous phenotypic adjustments in response to variations in light intensity, referred to as photoacclimation. The upper and lower limits of photosynthetic optimisation (photoacclimatory potential) can vary considerably between different Symbiodiniaceae species (Iglesias-Prieto and Trench 1994, 1997; Reynolds et al. 2008) or even between sister strains of the same species (Suggett et al. 2007, 2015; Díaz-Almeyda et al. 2017). Studies of symbionts in the coral *Goniastrea aspera* demonstrated that shaded surfaces experience chronic photoinhibition have lower recovery potential and are more prone to bleaching when exposed to elevated irradiance and temperatures (Brown et al. 2000a, 2000b, 2002). As such, it is plausible that disparities in photoacclimatory potential between symbionts may be the trait responsible for structuring symbiont communities across coral colonies.

Here, we characterise Symbiodiniaceae photochemical signatures of high-light and low-light niches of individual coral colonies, and test whether the disparity between high-light and low-light acclimation correlates with Symbiodiniaceae genetic disparity and multiple symbiont cohabitation. We hypothesised that the dynamic range of optical niches will vary across coral species, and that disparities in photoacclimation states will correlate with symbiont community compositional changes across the colony surface.

Methods

Sampling location and coral collection

Over an eight-day period, 100 coral colonies were sampled from the shallow (<2 m depth) lagoon of Rarotonga (September 2019, Cook Islands; ~21°12′7.92″S, 159°45′30.65″W). A diverse range of 20 coral species spanning 11 genera were selected according to their prevalence (Table 1; Supplementary Fig. 1, Supplementary table 1); these species spanned multiple colony morphologies, including branching (corymbose, digitate, hispidose, and closed), tabulate, massive, and encrusting growth forms (Veron 2000). Two small coral fragments (approximately 1.5 cm²) were collected using a chisel from each colony, one each from exposed and shaded regions (n = 200 total samples). Exposed and shaded regions were defined by their apparent light-exposed (i.e. upward facing at the colony apex) or shaded (i.e. underhanging or downwards facing) position. Following collection, samples were immediately transported to an on-site aquarium (30 L) where they were maintained

Table 1 Taxonomy of all coral species sampled from Rarotonga, Cook Islands. Specimens were identified based on comparison to type material, qualifiers follow the recommendations of (Cowman et al. 2020), and the names used are current according to the World Register of Marine Species (Hoeksema and Cairns 2021)

Family	Genus	Qualifier	Species	Growth form	Authority	*n* colonies sampled
Acroporidae	*Acropora*		*verweyi*	Corymbose	Veron & Wallace 1984	5
Acroporidae	*Acropora*	cf	*nasuta*	Corymbose	Dana 1846	5
Acroporidae	*Acropora*	cf	*globiceps*	Digitate	Dana 1846	5
Acroporidae	*Acropora*	aff	*valida*	Digitate	Dana 1846	5
Acroporidae	*Acropora*	sp.	1	Hispidose		5
Acroporidae	*Acropora*	sp.	2	Corymbose		5
Acroporidae	*Acropora*		*hyacinthus*	Tabular	Dana 1846	5
Agariciidae	*Pavona*	cf	*duerdeni*	Massive	Vaughan 1907	5
Euphyllidae	*Galaxea*	cf	*fascicularis*	Massive	Linneaus 1767	5
Incertae sedis	*Leptastrea*		*purpurea*	Encrusting	Dana 1846	5
Merulinidae	*Hydnophora*	cf	*microconos*	Massive	Lamarck 1814	5
Merulinidae	*Leptoria*		*phrygia*	Massive	Ellis & Solander 1786	5
Merulinidae	*Hydnophora*	cf	*exesa*	Encrusting	Pallas 1766	5
Merulinidae	*Astrea*		*curta*	Massive	Dana 1846	5
Merulinidae	*Echinopora*	sp.		Encrusting		4
Pocilloporidae	*Pocillopora*	cf	*meandrina*	Branching-Closed	Dana 1846	5
Pocilloporidae	*Pocillopora*	cf	*damicornis*	Branching-Closed	Linneaus 1758	4
Poritidae	*Porites*	cf	*lutea*	Massive	Milne Edwards & Haime 1860	5
Psammocoridae	*Psammocora*		*contigua*	Digitate	Esper 1794	5
Psammocoridae	*Psammocora*	sp.	2	Digitate		5

overnight with regular exchange of lagoon seawater and constant aeration.

Light utilisation capacity of exposed versus shaded regions

The photoacclimation state of each "exposed" or "shaded" fragment was estimated from fluorescence-light response curves of PSII photochemistry (MacIntyre et al. 2002). Active chlorophyll fluorescence was used to generate a rapid light curve (RLC) for each sample (Warner et al. 2010; Nitschke et al. 2018). The RLCs were produced using a Diving Pulse Amplitude Modulated (PAM) fluorometer (Walz GmbH, Effeltrich, Germany. Settings: MI: 6, Gain: 6, SI: 12, SW: 0.8 s, LC-INT: 3). To control for natural diel variation in the photo-physiological parameters, coral samples were subjected to RLCs in the first hour of daylight, when PSII fluorescence yields are expected to be maximal (Hill and Ralph 2005). The use of RLCs was preferred in this study to ensure all measurements were taken within a narrow time-frame, and we interpret the findings within the limitations of the method and not as absolute rates of electron transport in photosynthesis (Warner et al. 2010; Nitschke et al. 2018). Each RLC consisted of variable chlorophyll fluorescence yield measurements taken at nine discrete time-steps. A low-light acclimated (c.a. 5 µmol photons m^{-2} s^{-1}) measurement of the minimum and maximum fluorescence yields (F_o and F_m, respectively) initiated the RLC, followed by measurements under actinic light (F' and F_m') at eight 30 s intervals of increasing light intensity (61, 91, 126, 214, 320, 503, 801, and 1409 µmol photons m^{-2} s^{-1}). These parameters were used to estimate the maximum ($F_v/F_m = [F_m—F_o]/F_m$) and effective ($F_q'/F_m' = [F_m' − F']/F_m'$) photochemical efficiencies of the Symbiodiniaceae cells in each sample.

Symbiodiniaceae photoacclimate to specific light environments by changing their saturating irradiance (E_k), which represents the transition between the light-limited and the light-saturated states of PSII, accompanied by changes to the maximum photochemical efficiency of PSII ($F_q'/F_m'_{(max)}$) which typically increases under low-light conditions (Anthony and Hoegh-Guldberg 2003; Hennige et al. 2009). To estimate these parameters, the PE curves were fitted to a model (Eq. 1) that describes E_k and $F_q'/F_m'_{(max)}$ (Hennige et al. 2008).

$$F_q'/F_m' = \left[\left(F_q'/F_{m\,(max)}' E_k \left(1 - \exp\left(-E/E_k\right)\right) \right) \right]/E \qquad (1)$$

Photoacclimatory adjustments also include shifts in the reliance on photochemical quenching and nonphotochemical quenching pathways (Robison and Warner 2006; Hennige

et al. 2009). To estimate capacity to dissipate excitation energy via fluorescence and pathways of energy transfer other than fluorescence, the extent of light-dependant photochemical quenching ([1 − C], Eq. 2) and nonphotochemical quenching ([1 − Q], Eq. 3) were calculated at the final step in the RLC (Nitschke et al. 2018), to capture the maximum extent of dynamic quenching for each sample as:

$$[1 − C] = \left(F'_m − F \right) / \left(F'_m − F'_o \right) \tag{2}$$

$$[1 − Q] = \left(F'_v − F'_m \right) / \left(F_v − F_m \right) \tag{3}$$

Following PAM measurements, diffuse reflectance measurements of each sample were measured with a USB4000 spectroradiometer (Ocean Optics) at 1 nm resolution (400 to 750 nm) to broadly characterise the sample optical properties (D'Angelo et al. 2008; Winters et al. 2009; Cohen and Dubinsky 2015). A fibre-optic cable attached to a pulsed-xenon lamp (PX-2, Ocean Optics) was used to deliver full-spectrum light, and reflectance delivered to the USB2000 measuring unit via a fibre bifurcation. Reference spectra of the light source (acquired from a tissue-free coral skeleton) and background noise (in darkness) were stored and each measurement was acquired with the optic positioned at a 45° angle to the coral surface, at approximately 5 mm distance. Three acquisitions were averaged to provide the final spectrum. Raw data were processed (wavelength limits: 400 nm—750 nm; locally weighted scatterplot smoothing with a parametrised span of 0.1), and the total diffuse reflectance, i.e. the sum of the relative reflectance between 400 and 750 nm, was calculated with the R package "pavo" (Maia et al. 2013).

DNA extraction, PCR amplification, and sequencing

After photobiology data collection, each sample was rinsed with 0.22 μm filtered seawater, blot-dried with paper towel, and transferred to a salt-saturated DMSO preservation buffer (Seutin et al. 1991). DNA was extracted from each sample using a CTAB-chloroform DNA extraction protocol (Cunning et al. 2016). Symbiodiniaceae diversity was determined through sequencing of the ITS2 region. The "ITSDino" (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTG AATTGCAGAACTCCGTG-3') (Pochon et al. 2001) and "ITS2-Rev2" (5'-GTCTCGTGGGCTCGGAGATGTGTA TAAGAGACAGCCTCCGCTTACTTATATGCTT-3') (Stat et al. 2009a) primers (including Illumina adaptor sequences) were used to amplify the ITS2 region in PCR. The optimised cycling conditions were denaturation for 3 min at 95 °C, followed by 25 cycles of 30 s at 95 °C, 30 s at 55 °C, 30 s at 72 °C, and a final extension for 5 min at 72 °C. PCR amplicons were then cleaned with AMPure XP beads following the manufacturer's protocol. Cleaned PCR amplicons were

normalised to 2 ng/μl for a second indexing PCR reaction with a Nextera XT Library Prep kit (Illumina) with a dual indexing strategy. To create the final library for sequencing, 5 μl of each indexed sample was pooled and the final library cleaned, again using the AMPure XP Bead protocol. A Bioanalyzer trace and Qubit were used to measure the nanomolarity of the final library, and it was sequenced at 5 pM on a 500 cycle MiSeq run with 15% PhiX spike-in.

To further explore Symbiodiniaceae diversity, the chloroplast psbA non-coding region (psbA^{ncr}) was amplified (a marker that carries phylogenetic resolution at the species to sub-species level in Symbiodiniaceae (LaJeunesse and Thornhill 2011; Thornhill et al. 2014)) for a subset of *Acropora* and *Psammocora* samples. Samples were selected based on the criterion of harbouring a single ITS2 profile (see below) in high abundance (> 80% in relative abundance of the sample total sequence count). The 7.4-For (5'-GCA TGAAAGAAATGCACACAACTTCCC-3') and 7.8-Rev (5'-GGTTCTCTTATTCCATCAATATCTACTG-3') primer pair was used as previously described (LaJeunesse and Thornhill 2011), and PCR products were Sanger sequenced in both directions (Macrogen, South Korea).

Bioinformatics

Primer sequences were removed from all reads using Cutadapt (Martin 2011) and the raw sequence data were denoised using the DADA2 pipeline to delineate sequencing errors from biological diversity (Callahan et al. 2016) following the author-recommended ITS2 workflow. DADA2 'amplicon sequence variants' (ASVs) were assigned a taxonomic identity using the RDP classifier (Wang et al. 2007) against a custom, previously published Symbiodiniaceae-enriched ITS2 reference database (github.com/nitschkematthew/ Symbiodatabaceae). The default RDP bootstrap value of 50 was used to assign taxonomic ranks to an ASV; below this threshold the rank was set to 'unassigned'. Non-Symbiodiniaceae sequences were removed at this point prior to any further analyses. To visualise the genetic diversity within *Cladocopium* across all samples, an alignment-free phylogenetic tree was generated using a k-mer (k = 7)-based clustering method [kmer, *kdistance* (Wilkinson 2018)].

To investigate Symbiodiniaceae diversity at a resolution that incorporates ITS2 intragenomic variation, the raw sequences (which are available online at the National Center for Biotechnology Information, Sequence Read Archive: BioProject number PRJNA863925) were submitted to SymPortal (Hume et al. 2019). SymPortal uses a minimum entropy decomposition (MED)-based approach to consolidate ITS2 sequences into nodes and catalogues novel post-MED sequences within the SymPortal ITS2 reference database. By comparing the assemblage of post-MED

sequences between each sample, SymPortal identifies co-occurring 'defining' intragenomic sequence variants (DIVs) which are used to designate ITS2 type profiles representative of putative Symbiodiniaceae taxa (Hume et al. 2019).

To validate the identification of the closely-related ITS2 profiles within the C3 and C1 radiations of *Cladocopium*, the chloroplast psbA^{ncr} marker was analysed on a subset of samples. The psbA^{ncr} chromatograms were visually inspected and trimmed according to the stretch of unambiguous base calls. Samples with any low-quality sequences, potentially due to intra- or inter-genomic variation, were discarded. Due to the length of psbA^{ncr} amplicons, the forward and reverse reads did not overlap for the generation of consensus sequences. Therefore, the forward and reverse reads for each sample were aligned separately [DECIPHER, *Alignseqs* (Wright 2016)] and then concatenated before a maximum parsimony tree was produced using RAxML (Stamatakis 2014) on the CiPRES portal (HPC v.8 on XSEDE (Miller et al. 2011)) using default settings. FASTA files containing the concatenated forward and reverse complement psbA sequences can be retrieved from the supplementary material.

Statistical analyses

To compare the photo-physiological characteristics and reflectance spectra of symbionts found within shaded and exposed microhabitats, a Kruskal–Wallis test (nonparametric ANOVA) was performed on $F_q'/F_m'_{(max)}$, E_k, $[1 - C]$, $[1 - Q]$, and total diffuse reflectance between microhabitats from samples pooled across all coral species.

To explore ITS2 sequence differentiation between shaded and exposed microhabitats, ASV accumulation curves were produced for exposed samples only, and then on paired (within-colony) shaded and exposed samples with pooled ASVs [Vegan, *specaccum* (Dixon 2003)]. Across all coral samples, the number of ASVs that were detected in only shaded samples, only exposed samples, and in both microhabitats were counted. DeSeq2 was used to test for the differential abundance of ASVs and post-MED sequences between shaded and exposed habitats (Love et al. 2014), with a Wald test on the dispersion around group means. To control the false discovery rate, the p-values for all differential abundance tests were adjusted together using the Benjamini–Hochberg method. To prevent multiple observations of nil sequence abundance from significantly skewing the group means, the DeSeq2 results were filtered to retain only ASVs and post-MED sequences that were detected in at least four samples within at least one habitat. A permutational multivariate analysis of variance (PERMANOVA) was performed on ITS2 profiles across the dataset to test for differences between coral species and habitats [Vegan, *adonis* (Dixon 2003)].

Comparisons of symbiont community diversity using the ITS2 marker are complicated by the fact that unique ITS2 ASVs are not necessarily equivalent to distinct species. Therefore, to capture between-sample sequence diversity, we utilised the weighted UniFrac method to provide a phylogenetically-informed distance metric (Lozupone and Knight 2005). Coral species that had at least one differentially abundant sequence (i.e. significant within-colony genetic heterogeneity) were selected for further analyses. A phylogenetic tree was generated for ASV sequences using *k*-mer clustering [kmer, *kdistance* (Wilkinson 2018)] and a weighted UniFrac distance matrix was computed in phyloseq for each coral species [phyloseq, *distance*, McMurdie and Holmes (2013)]. For each coral species, a linear regression between the within-colony weighted UniFrac distance values and the within-colony fold difference in functional photobiological parameters (E_k, $F_q'/F_m'_{(max)}$, $[1 - C]$, $[1 - Q]$) was performed. All linear regressions were corrected for multiple tests using the Benjamini–Hochberg method. From the DeSeq2 outputs and SymPortal ITS2 data, it was apparent that sequence abundance differences between optical habitats are common for rare sequences. Therefore, each sample was filtered for ASVs that represented < 1% of the total sample read count (a threshold used to define the "rare microbial biosphere"; (Boulotte et al. 2016)), and the weighted UniFrac distances between sample pairs were again correlated with the fold difference in photobiological parameters. All code required to reproduce data processing and analysis can be found at https://github.com/Robert-Lewis-NZ/Rarotonga_2019_shaded_vs_exposed.

Results

Photophysiology

Photosynthetic acclimation states differed between shaded and exposed habitats for all parameters: $F_q'/F_m'_{(max)}$ ($\chi^2 = 80.971$, p < 0.001); E_k ($\chi^2 = 43.07$, p < 0.001); $[1 - C]$ ($\chi^2 = 68.74$, p < 0.001); $[1 - Q]$ ($\chi^2 = 49.7$, p < 0.001); and total diffuse reflectance ($\chi^2 = 71.9$, p < 0.001; Fig. 1). On average, shaded samples exhibited 13% higher $F_q'/F_m'_{(max)}$ and 50% lower E_k values than exposed samples; however, the fold differences varied considerably across the coral species (for species specific parameter estimates, see Supplementary Figs. 2–5). The mean maximal photochemical quenching coefficient $[1 - C]$ of shaded samples was 47% lower than exposed samples, indicating greater reliance on photochemical quenching. Correspondingly, the mean maximal nonphotochemical coefficient $[1 - Q]$ of shaded samples was 16% higher than exposed samples, indicating less reliance on nonphotochemical quenching. The mean total
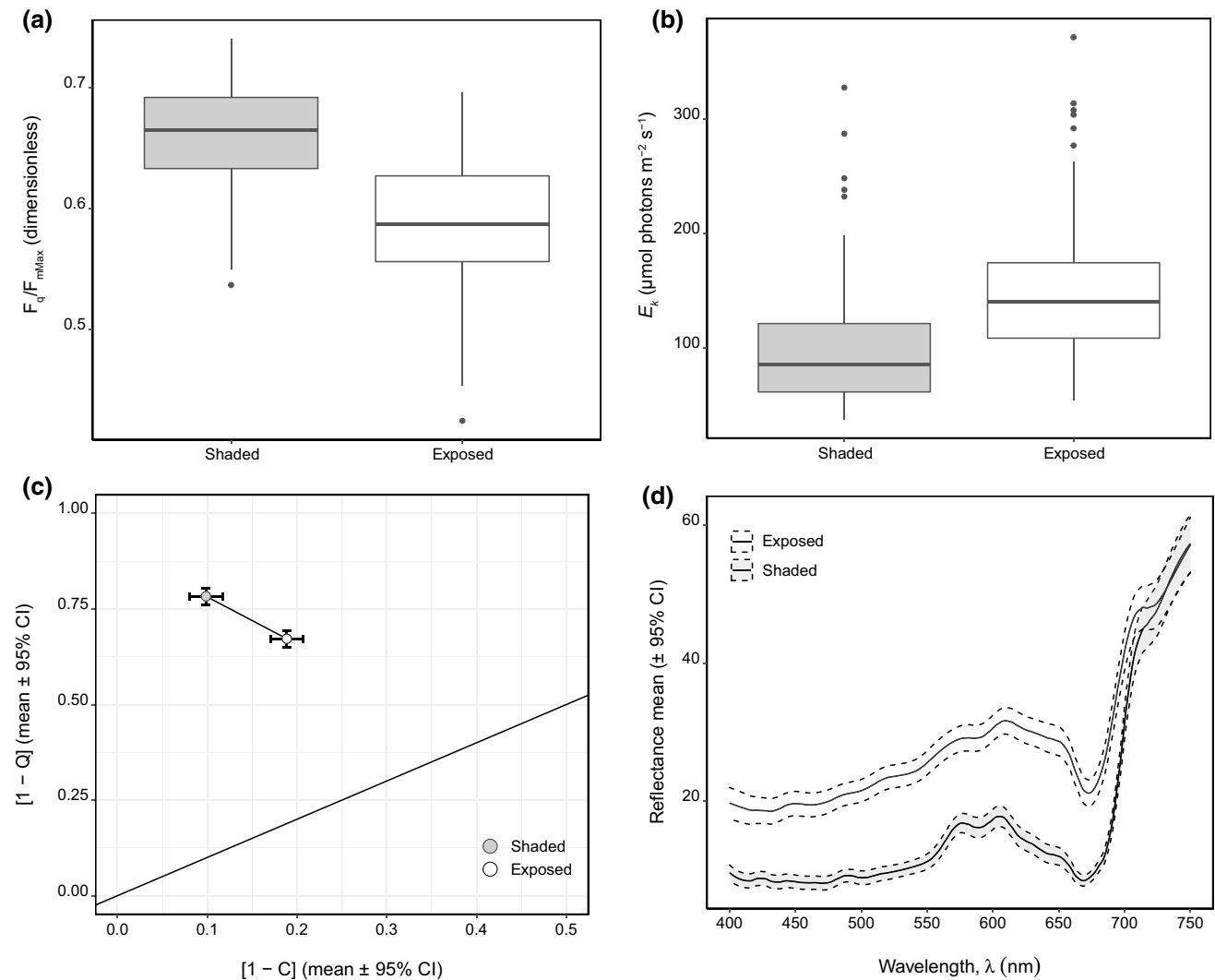
(a)

(b)

(c)

(d)

Fig. 1 Signatures of low and high light acclimation of Symbiodiniaceae across all coral species. Boxplots of **A** Maximum photochemical efficiency of PSII ($F_q'/F_m'_{(max)}$) values and **B** minimum saturating irradiance (E_k) in shaded (grey) and exposed (white) samples. C) Mean maximal photochemical quenching [1 − C] and nonphotochemical quenching [1 − Q] coefficients of shaded (black symbol) and exposed (white symbol) samples ($\pm 95\%$ CI). The [1 − C]:[1 − Q] = 1 slope is depicted as a solid line. D) Mean reflectance of shaded (grey) and exposed (white) samples between 400 and 750 nm (dashed line above and below mean represent $\pm 95\%$ CI). N = 98 per microhabitat type in all plots, integrating all coral species

diffuse reflectance of exposed samples was 70% greater than shaded samples (Fig. 1).

Symbiodiniaceae ITS2 sequence diversity

Illumina ITS2 sequencing yielded 9,136,599 Symbiodiniaceae sequences after quality filtering and the removal of chimeric sequences. Two sample pairs (one from *Pocillopora* cf. *damicornis* and one from *Echinopora* sp.) were removed from the dataset due to low read counts. The total number of reads *per* sample averaged 46,615 ± 17,472 (SD). A total of 909 Symbiodiniaceae ASVs were assigned to four Symbiodiniaceae genera: *Cladocopium*, *Durusdinium*,

Symbiodinium, and *Breviolum,* which were present within the dataset at a relative frequency of 0.856, 0.144, 0.0004, and 0.0001 of the total reads, respectively. The majority of ASVs were assigned to seven distinct radiations within *Cladocopium* (C1, C1c, C42, C15, C3, C3*, and C3k; Supplementary Fig. 6). Within-colony pooling of shaded and exposed sequences together provided 155 more distinct ASVs compared with exposed samples only across the community of 20 coral species (Fig. 2).
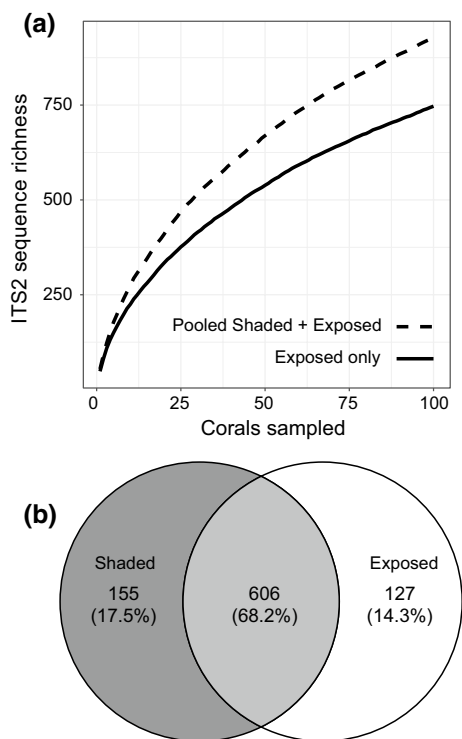
Fig. 2 Symbiodiniaceae ITS2 sequence differences between shaded and exposed samples. **A** Accumulation curves of Symbiodiniaceae ITS2 (ASV) sequences for paired (within-colony) shaded and exposed samples pooled together (dashed line) and exposed samples only (solid line). The difference between the accumulation curves represents increasing evidence for symbiont niche differentiation as more colonies are sampled. **B** The total number of ITS2 ASVs exclusively detected in shaded samples only (left), exposed samples only (right), and in both microhabitats (centre)

Differential abundance of ITS2 sequences

No single ASV was found to be significantly differentially abundant in either habitat when all samples were pooled together, likely due to high levels of host-specificity. However, when analysed within each coral species, nine ASVs and seven post-MED sequences were significantly differentially abundant with two ASVs being consistently more abundant within exposed habitats across three coral species (Fig. 3). For most species, the differentially abundant ASVs were rare in relative abundance, except for *Porites* cf. *lutea*, where differentially abundant ASVs comprised up to 80.6% of the total reads. Excluding *Porites* cf. *lutea*, the mean relative frequency of all differentially abundant ASVs and post-MED sequences collectively pooled within each species was 0.031 ± 0.035 and 0.033 ± 0.056 (SD), respectively. Three (43%) of the differentially abundant post-MED sequences were DIVs within *Cladocopium* ITS2 profiles, and four (57%) were not DIVs in this dataset or any previous datasets within the SymPortal database (at the time of analysis).

ITS2 profile diversity: SymPortal analysis

SymPortal yielded a total of 8,991,072 post-MED Symbiodiniaceae sequences across all samples and assigned these sequences to the genera *Cladocopium*, *Durusdinium*, *Symbiodinium* and *Breviolum*. From these sequences, 31 ITS2 DIV profiles were generated, of which DIVs represented 83.4 ± 7.7 (SD) of the sequence counts within each sample (Fig. 4). PERMANOVA analyses of ITS2 profile composition revealed that Symbiodiniaceae assemblages varied
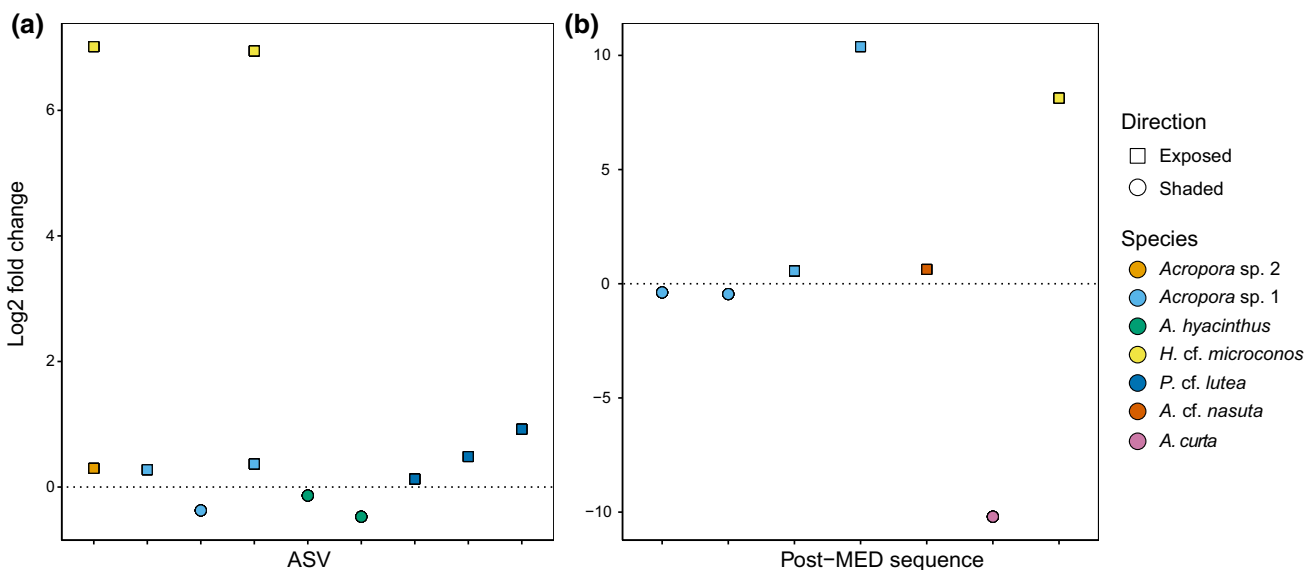

Fig. 3 Differential abundance of Symbiodiniaceae ITS2 sequences between shaded and exposed samples within each coral species. Log2 fold change in ASV abundance for significantly differentially abun-

dant **A** DADA2 ASVs and **B** SymPortal post-MED sequences that are more abundant in exposed (square symbols) or shaded (circle symbols) samples (Benjamini–Hochberg adjusted $p < 0.05$)

Fig. 4 ITS2 DIV profiles of exposed and shaded coral samples. Pairs of horizontal bars represent matching exposed (left) and shaded (right) samples from a single coral colony. Each colour indicates a unique ITS2 DIV profile inferred by SymPortal. Symbiodiniaceae sequences not assigned to the sample profiles are depicted in grey
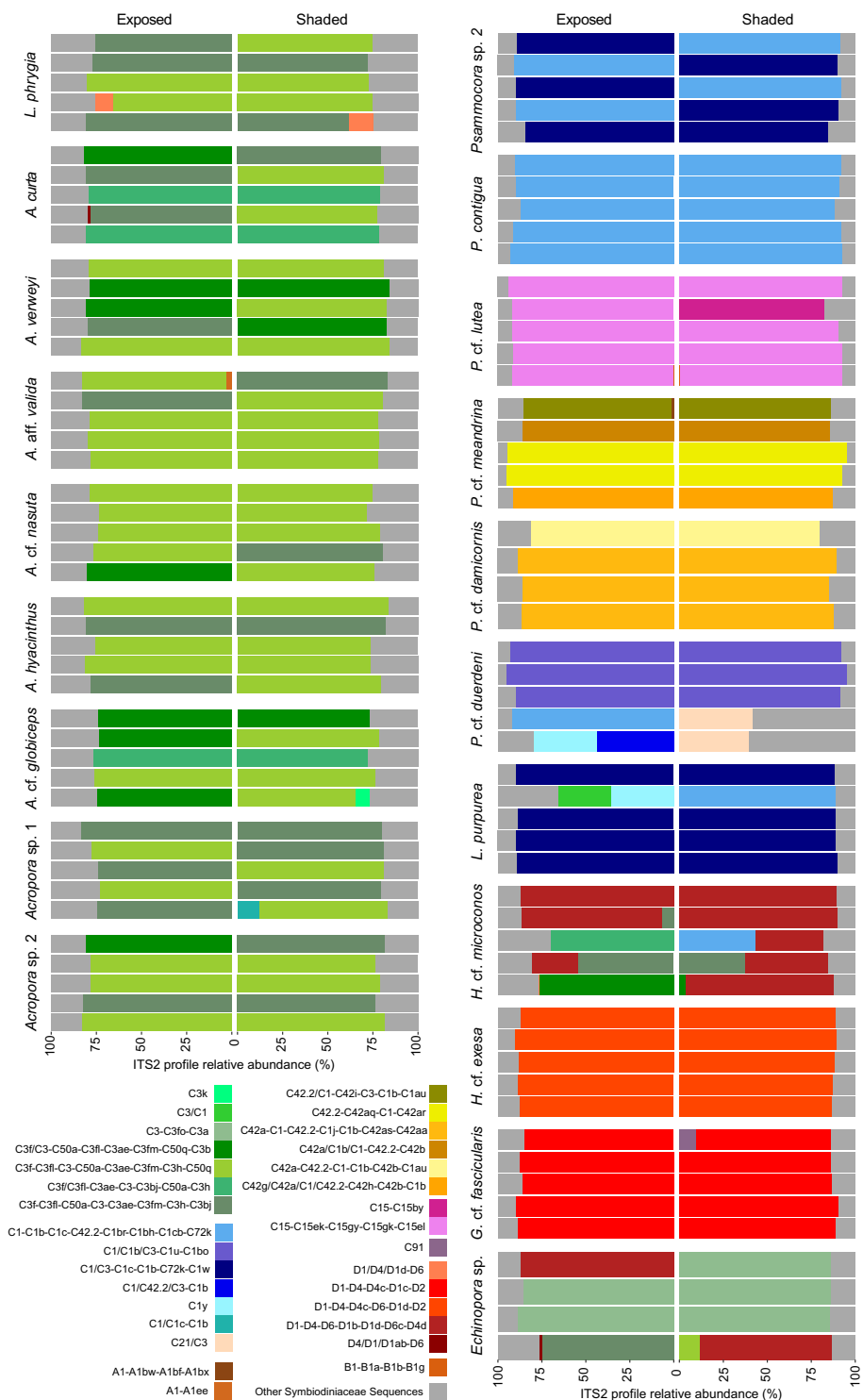
significantly by species (F = 9.16, p = 0.001), but neither habitat nor the interaction between species and habitat had a significant effect on profile composition.

However, several coral-specific patterns in the distribution of ITS2 profiles were evident. All samples from each of *Hydnophora* cf. *exesa*, *Galaxea* cf. *fascicularis*, and *Psammocora contigua*, regardless of habitat,

contained one profile (D1-D4-D4c-D6-D1d-D2, D1-D4-D4c-D1c-D2, and C1-C1b-C1c-C42.2-C1br-C1bh-C1cb-C72k, respectively). Four DIV profiles from the C3 radiation were found to be randomly distributed across the *Acropora* spp. in both habitats, all of which were also found in *Hydnophora* cf. *microconos*, *Astrea curta*, *Echinopora* sp., and *Leptoria phrygia*. When the C3f/

C3fl-C3ae-C3-C3bj-C50a-C3h profile was observed in *A. curta* and *Acropora* cf. *globiceps*, it was dominant in both shaded and exposed habitats. Additional C3 majority sequence profiles not found in *Acropora* were present in *Echinopora* sp. and *Leptastrea purpurea*. Six profiles from the C42 radiation were found in the pocilloporids, with each colony dominated by a single profile.

Psammocora sp. 2, *L. purpurea*, *Pavona* cf. *duerdeni*, *Echinopora* sp., and *Hydnophora* cf. *microconos* each exhibited within-colony differentiation of ITS2 profiles. Two C1 majority sequence profiles dominated the colonies of *P. contigua* and alternated between exposed and shaded depending on the colony. One *L. purpurea* colony contained three distinct profiles; C3 and C1 majority sequence profiles co-dominated the exposed sample, while a distinct C1 majority sequence profile dominated the shaded sample. Two *P. duerdeni* colonies harboured two and three distinct profiles each; C1 majority sequence profiles dominated the exposed samples, whereas a single DIV profile (C21) occupied the shaded samples of both colonies. Half of the *E. gemmacea* colonies had differentiated profile compositions; C3 and D1 majority sequence profiles dominated the exposed and shaded samples of one colony, respectively, whilst this pattern was reversed in the other colony. Four *H. microconos* colonies harboured two or three distinct profiles *per* colony; C3 majority sequence profiles were more abundant in exposed samples, whereas C1 and/or D1 majority sequence profiles dominated the shaded samples (Fig. 4).

psbA^{ncr} sequence diversity

The psbA^{ncr} alignments for samples containing C3 or C1 ITS2 majority sequence profiles contained multiple blocks of indels. Phylogenetic analysis of nucleotide substitutions revealed that the C3f/C3fl-C3ae-C3-C3bj-C50a-C3h profile formed a divergent clade to the other C3 majority sequence profiles found in *Acropora* (Supplementary Fig. 7), consistent with C3f/C3fl-C3ae-C3-C3bj-C50a-C3h co-occurrence in both exposed and shaded samples of single colonies. The two C1 majority sequence profiles (C1-C1b-C1c-C42.2-C1br-C1bh-C1cb-C72k and C1/C3-C1c-C1b-C72k-C1w) were not congruent with psbA^{ncr} diversity.

ITS$_2$ sequence diversity and photo-physiology

When analysed on the complete sample ASV diversity, no linear regressions between the paired-sample difference in the photobiological parameters and UniFrac distance were significant. However, after filtering for only rare sequences (<1%) within each sample, *H.* cf. *microconos* displayed a strong positive correlation between the paired-sample difference in extent of $[1 - Q]$ and the ASV UniFrac distances (Fig. 5), suggesting that rare sequences are diagnostic of
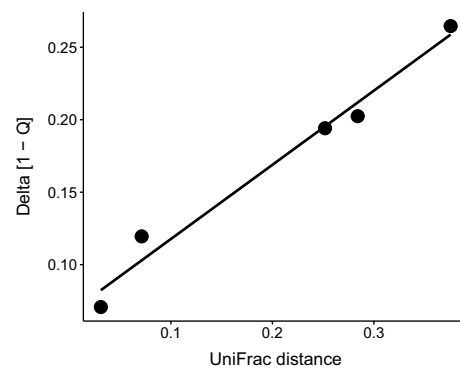

Fig. 5 Photosynthetic signatures that correlate with Symbiodiniaceae genetic disparity. Within-colony difference in $[1 - Q]$ utilisation *versus* within-colony genetic disparity (weighted unifrac distance) of rare (<1%) Symbiodiniaceae sequences in *Hydnophora* cf. *microconos*. Points represent individual *H.* cf. *microconos* colonies. The line represents the linear regression fit to data (F = 11.2, Benjamini–Hochberg adjusted $p = 0.042$)

functionally distinct symbiont communities or genotypes within a single colony.

Discussion

Five distinct but interrelated findings are presented here which provide insights into within-colony partitioning of Symbiodiniaceae. First, symbiont communities inhabiting shaded and light exposed coral tissues consistently demonstrate photobiological signatures of acclimation or adaptation to 'low' and 'high'-light exposure, respectively. Second, pooling shaded and exposed samples yielded an additional 155 ITS2 sequences compared to exposed samples alone, suggesting this sampling design can serve as a new standard for the retrieval of Symbiodiniaceae genetic diversity. Third, multiple coral species have differentially abundant background Symbiodiniaceae ITS2 sequences, suggesting that the rare symbiont biosphere may exhibit substantial community differentiation between optical niches, or that rare sequence variants may be diagnostic of strain-level partitioning. Fourth, multiple coral species exhibited distinct ITS2 profiles in shaded *versus* exposed regions of individual colonies, suggesting that symbionts competing within a colony can become locally dominant via optical niche partitioning, and this may be more common on coral reefs than realised. Fifth, for *Hydnophora microconos*, the within-colony UniFrac distances of rare (<1%) Symbiodiniaceae sequences were strongly positively correlated with the difference in $[1 - Q]$ utilisation, suggesting that differences in functional capacity may underpin symbiont community differentiation. These lines of evidence support the notion that within-colony light gradients influence Symbiodiniaceae

species cohabitation, highlighting a need for an approach to symbiont ecology from the perspective of coral colonies as heterogenous habitats.

Shaded and exposed tissues yield distinct and differentially abundant sequences

Dividing coral colonies into regions defined as shaded or light exposed revealed Symbiodiniaceae genetic disparity. In most cases, the collective abundance of the differentially abundant sequences was relatively low, suggesting that rare symbionts are regulated by light availability to a greater extent than dominant symbionts. Although background Symbiodiniaceae sequences are frequently detected in deep-sequencing analyses (Arif et al. 2014; Quigley et al. 2014; Thomas et al. 2014; Hume et al. 2020), the factors regulating the prevalence and diversity of rare symbionts remain largely unresolved (Boulotte et al. 2016; Lee et al. 2016). The limited research to date suggests that rare symbionts may be more sensitive or responsive to environmental change than dominant symbiont species. For example, Boulotte et al. (2016) profiled the ITS2 diversity in two coral species before and after two separate bleaching events and found that novel rare Symbiodiniaceae sequences were detected after each bleaching episode. Similarly, Lee et al. (2016) found that the presence of background Symbiodiniaceae appeared to fluctuate substantially throughout an annual cycle. Differentially abundant sequences could also represent strain-level intragenomic variation among dominant symbionts (LaJeunesse et al. 2010; Arif et al. 2014), with each strain occupying a different region in the colony. Most (57%) of the differentially abundant post-MED sequences of this study did not belong to an ITS2 profile and were not DIVs of any other ITS2 profiles in the SymPortal reference database, suggesting that these environmentally responsive Symbiodiniaceae species (or intragenomic variants) are consistently rare. Assigning these rare sequences to genomes and developing technologies to visualise these cells within coral tissues (e.g. via fluorescence in situ hybridisation (Yokouchi et al. 2003)) will be essential to measure the functional significance of their physiologies and the adaptations that enable them to persist in the presence of abundant competitors.

Symbiont cohabitation across microhabitats via the analysis of ITS2 profiles

Several coral species, including *H.* cf. *microconos*, *P.* cf. *duerdeni*, *Echinopora* sp., and *L. purpurea*, displayed marked within-colony differences in the dominant ITS2 profiles, including examples of both *Cladocopium-Cladocopium* and *Cladocopium-Durusdinium* cohabitation. Within-colony partitioning of Symbiodiniaceae species into optical niches where they become the locally dominant symbiont

may be more common in coral communities than previously realised. However, within these examples, the dominant symbiont community was not always differentiated by light exposure. Competitive hierarchies and successional processes may cause adult colonies to become almost entirely dominated by a single competitively-superior symbiont species (Abrego et al. 2009; McIlroy et al. 2019), despite the potential for cohabitation with additional symbiont species. Although competitive hierarchies may depend on the prevailing light conditions (McIlroy et al. 2019), it seems plausible that coral-symbiont coevolution may produce partnerships that function optimally under a wide range of conditions (Gabay et al. 2019). In turn, these optimal partners may exclude other symbiont species from establishing, producing a relatively homogeneous symbiont community among individuals of a given coral species.

Morphological variation between colonies of the same species may cause within-colony light gradients to be more pronounced for some individuals (as evidenced by the variation in the fold differences of photobiological parameters within each species; Supplementary Figs. 2 and 3). It is notable that the four coral species with differentiated symbiont communities had either massive or encrusting morphologies, and spatial differentiation of symbionts has been observed among these growth forms in past studies (Garren et al. 2006; Kemp et al. 2008, 2014). These skeletal morphologies may be more likely to form overhanging protrusions or cervices and self-shade compared to branching morphologies which are considered more effective at redistributing light throughout the colony via diffuse scattering compared to massive skeletons (Marcelino et al. 2013; Enríquez et al. 2017). Ultimately, establishing a causal link between coral bio-optics and symbiont niche differentiation will require a quantitative spatial framework that integrates the three-dimensional complexity of individual corals and the corresponding gradients of incident light.

psbA[ncr] sequences hint at Cladocopium strain-level differentiation

Several psbA[ncr] *Cladocopium* haplotypes were recovered (containing substitutions and/or insertions/deletions) that were not congruent with the corresponding ITS2 DIV profile. This may be indicative of strain-level genetic variation in which an assortment of *Cladocopium* strains of a single species are distributed heterogeneously between and within coral colonies (see also Thornhill et al. 2014). However, psbA[ncr] haplotypes assigned to the C3f/C3fl-C3ae-C3-C3bj-C50a-C3h and C3f-C3fl-C50a-C3-C3ae-C3fm-C3h-C3bj ITS2 DIV profiles formed distinct clades, potentially representing a distinct, but closely related *Cladocopium* species. This highlights the power of aligning SymPortal outputs with the psbA[ncr] marker for delineating Symbiodiniaceae

species (Smith et al. 2020). Interestingly, across both *A.* cf. *globiceps* and *A. curta*, the C3f/C3fl-C3ae-C3-C3bj-C50a-C3h ITS2 profile completely dominated both microhabitats of a colony whenever it was present. This could be evidence of a species- or strain-level competitive hierarchy where certain taxa with wide photoacclimatory potentials outcompete other Symbiodiniaceae from all optical niches when they establish within a colony. Over long timescales, such competitive hierarchies could explain why some Symbiodiniaceae eventually reach near ubiquitous representation across many 100's of kms of reef (Pettay and Lajeunesse 2013).

Hydnophora microconos as a new model species to study symbiont cohabitation

H. cf. *microconos* was particularly intriguing due to the inter- and intra-genus diversity of their dominant Symbiodiniaceae. *Hydnophora* spp. are known to acquire their symbionts horizontally (Fabina et al. 2012) and *H. microconos* has previously been reported to associate with either *Cladocopium* or *Durusdinium* on the same reef (LaJeunesse et al. 2003). We propose that *H. microconos* may serve as an ideal model species for further investigations of symbiont cohabitation and may be considered as a Pacific analogue to the model coral species *Orbicella faveolata* in the Caribbean (Kemp et al. 2014). Inherent symbiont flexibility may enhance the potential for colonisation of a cohort of symbiont species [potentially during the recruitment phase (Quigley et al. 2017)] that subsequently become partitioned across multiple optical niches as the colony grows. Indeed, symbiont flexibility may be integral for allowing symbiont species to occupy distinct optical niches in perpetuity.

In *H.* cf. *microconos*, the extent of phylogenetic distance among low-abundance sequences across shaded and exposed fragments within a single colony was strongly positively correlated with the magnitude of difference in nonphotochemical quenching utilisation $[1 - Q]$. The capacity to preferentially dissipate excitation energy via $[1 - Q]$ is an important trait for tolerating light stress and is known to broadly correlate with Symbiodiniaceae genera (Robison and Warner 2006; Suggett et al. 2015). However, preferential nonphotochemical quenching reliance can also vary between isolates of the same species, highlighting the capacity of Symbiodiniaceae to adapt to differing environmental histories (Howells et al. 2012; Suggett et al. 2015). Differences in rare symbiont diversity between microhabitats may be partially driven by the selection for symbionts with a high capacity for nonphotochemical quenching in exposed colony regions. However, rare symbionts likely do not contribute strongly to the fluorescence signal of the PAM and, therefore, it is not possible to discern if these rare symbionts share this phenotype. The extent of nonphotochemical quenching in dominant symbiont taxa may serve as diagnostic of distinct optical niches

and a proxy for zones likely harbouring diverse background symbiont species.

Conclusion

Due to the rapid nature of climate change, coral reef resilience may largely depend on capacity for adaptive shifting of pre-existing symbiont diversity within each colony (Cunning et al. 2015; Silverstein et al. 2015). Predicting rearrangements in symbiont species may require a theoretical framework that incorporates differential symbiont performance, competition, changing niche spaces, and successional processes within the holobiont, i.e. an ecology of Symbiodiniaceae interactions within corals. Gradients of irradiance across coral colonies may partially explain the coexistence of diverse symbiont taxa, and our data suggest that pooling sequences from exposed and shaded niches of individual colonies is an effective method for capturing the extent of within-colony Symbiodiniaceae diversity. However, future studies are needed to answer whether sampling colony extremities are sufficient to capture total within-colony Symbiodiniaceae diversity, or whether multi-variable niches (e.g. light × water flow/nutrient exchange combinations) promote additional diversity. Niche-partitioning may enhance the genomic variation available within the host, strengthening the ability of corals to maintain their adaptive potential. Validating the light-driven niche-differentiation hypothesis will ultimately require more observational studies and experimental manipulation of coral colonies. As corals face increasingly threatening circumstances globally, symbiont niche-differentiation should promptly inspire further investigation.

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