

FEATURE ARTICLE



Mangrove lagoons of the Great Barrier Reef support coral populations persisting under extreme environmental conditions

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ABSTRACT: Global degradation of coral reefs has increased the urgency of identifying stress-tolerant coral populations, to enhance understanding of the biology driving stress tolerance, as well as identifying stocks of stress-hardened populations to aid reef rehabilitation. Surprisingly, scientists are continually discovering that naturally extreme environments house established coral populations adapted to grow within extreme abiotic conditions comparable to seawater conditions predicted over the coming century. Such environments include inshore mangrove lagoons that carry previously unrecognised ecosystem service value for corals, spanning from refuge to stress preconditioning. However, the existence of such hot-spots of resilience on the Great Barrier Reef (GBR) remains entirely unknown. Here we describe, for the first time, 2 extreme GBR mangrove lagoons (Woody Isles and Howick Island), exposing taxonomically diverse coral communities (34 species, 7 growth morphologies) to regular extreme low pH (<7.6), low oxygen (<1 mg l⁻¹) and highly variable temperature range (>7°C) conditions. Coral cover was typically low (<5%), but highly patchy and included established colonies (>0.5 m diameter), with net photosynthesis and calcification rates of 2 dominant coral species (*Acropora millepora*, *Porites lutea*) reduced (20–30%), and respiration enhanced (11–35%), in the mangrove lagoon relative to adjacent reefs. Further analysis revealed that physiological plasticity (photosynthetic ‘strategy’) and flexibility of Symbiodiniaceae taxa associations appear crucial in supporting coral capacity to thrive from reef to lagoon. Prevalence of corals within these extreme conditions on the GBR (and elsewhere) increasingly challenge our understanding of coral resilience to stressors, and highlight the need to study unfavourable coral environments to better resolve mechanisms of stress tolerance.



Acropora millepora colony thriving under extreme low pH, low oxygen and highly variable temperatures of a Great Barrier Reef mangrove lagoon.

Photo: Emma Camp

KEY WORDS: Coral reefs · Extreme environments · Mangroves · Climate change · Marginal environments · Symbiodiniaceae · Warming · Ocean acidification

1. INTRODUCTION

Progressive decline of coral cover throughout the Great Barrier Reef (GBR) since at least the 1980s (Sweatman et al. 2011, De'ath et al. 2012) has now been superseded by catastrophic coral mortality in just 2 yr, from the worst-ever recorded mass coral bleaching (>30%, 2016–2017; Hughes et al. 2017, 2018). These bleaching events have already led to

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declines in coral reproductive capacity (Hughes et al. 2019) and reef accretion (McMahon et al. 2019), compromising the potential resilience of the reef to the anticipated increase in frequency and intensity of bleaching events (Oliver et al. 2018). While environmental filtering of thermally tolerant corals surviving after bleaching events will naturally select for some thermally resilient taxa, reductions in coral species that have traits supporting key ecological functions, e.g. reductions in species contributing to habitat provision and rapid reef accretion (Hughes et al. 2018), creates a need to identify and study potential hot-spots of coral resilience where these species will be supported. Such hot-spots of resilience include both coral refugia (locations maintaining favourable conditions being lost elsewhere, sensu Keppel & Wardell-Johnson 2012; e.g. the northern Red Sea, Osman et al. 2018) and habitats housing intrinsically resilient coral populations (reviewed by Camp et al. 2018a).

To date, studies of coral refugia environments documented on the GBR have focussed on environments that dampen the effects of heat stress; for example, microrefugia operating over small scales appeared to enable stress-sensitive *Acropora* coral populations to persist through the 2016–2017 marine heatwaves (Hoogenboom et al. 2017). Bleaching models have also predicted that the southern end of the GBR may act as a temporary coral refugium (van Hooidek et al. 2013). However, with current rates of ocean warming (IPCC 2014), and the increased frequency of marine heatwaves (Oliver et al. 2018), it remains unclear how long this geographic thermal refuge may operate. Deep reefs of the GBR were also documented to provide transient refuge to corals during the 2016–2017 bleaching event (Frade et al. 2018). Although incidence of coral bleaching was lower at deep reef sites (40 m), the cold water associated with the refugia resulted from seasonal oceanographic processes, temporally constraining the refuge capacity (Frade et al. 2018). These findings are consistent with other global upwelling refugia sites, where such temporal refuge restrictions operate (e.g. Chollett et al. 2010). In contrast, more temporally stable refugia on the GBR are inshore, turbid reefs. Inshore reefs of the GBR attenuate solar irradiance that otherwise can exacerbate thermal stress, resulting in reduced bleaching severity and sustained coral cover post-bleaching (Morgan et al. 2017). While refugia will contribute to aiding some coral populations through periods of stress, their temporal and spatial restrictions, combined with the fact that most refugia only offset a single stressor (reviewed by Camp et al. 2018b), means that there is arguably a more time-critical need to identify and

study naturally resilient coral populations in order to (1) understand which coral species can best survive multiple stressors, (2) identify the key mechanisms that support coral survival and (3) reveal any associated biological and ecological costs to corals with enhanced stress tolerance. Such knowledge can aid decisions that currently need to be made regarding which species should be targeted for restoration and adaptive management options (e.g. the GBR; Suggett et al. 2019), while highlighting potential risks associated with biodiversity and reef services.

Studies from natural extreme reef environments, such as carbon dioxide vents (e.g. Fabricius et al. 2011, Enochs et al. 2015), back reef tide pools on Ofu Island (Smith et al. 2007, Oliver & Palumbi 2009, 2011a) and mangrove lagoons (Camp et al. 2016a, 2017) have revealed that phenotypic plasticity, particularly in traits related to energy acquisition, are important in supporting coral survival (Camp et al. 2018b). For example, under naturally acidified waters, corals continue to calcify through increased photosynthesis rates to meet the required energy demands (Strahl et al. 2015). Changes in dominant algal symbiont (e.g. Oliver & Palumbi 2011b) and increased photosynthetic efficiency per cell of Symbiodiniaceae (Camp et al. 2016b) have also been shown to facilitate coral survival in naturally variable in-shore reef habitats. In mangrove lagoons, corals also appear to enhance respiration rates, indicative of increased heterotrophy, which may offset losses to photosynthetic capacity and therefore facilitate their survival (Camp et al. 2017). Mangrove lagoons are of particular interest because they consistently expose resident coral populations to low pH, low oxygen and warmer waters relative to adjacent reefs (Seychelles, Indonesia, Camp et al. 2016a; New Caledonia, Camp et al. 2017), i.e. the primary trio of stressors predicted to impact coral reefs under climate change (IPCC 2014, Camp et al. 2018b). For example, in New Caledonia, corals in the Bouraké mangrove lagoon were regularly exposed to pH below 7.4, oxygen below 2 mg l^{-1} , and temperature 2°C higher than the adjacent reef (Camp et al. 2017). Whether mangrove lagoons of the GBR have similarly extreme abiotic conditions and house abundant and/or diverse coral communities remains entirely unknown. Furthermore, if these locations exist, whether resident coral populations employ plasticity in energy acquisition modes and conform with observations reported from other extreme inshore coral habitats is also unknown.

Here we surveyed 250 km of the northern GBR, visiting lagoons located on 5 off-shore islands. From this exploration, we identified for the first time 2 man-

grove lagoons with relatively extreme environmental conditions (low pH, low oxygen and highly varying temperature), coupled to significant coral cover and/or diversity. Targeted studies of resident corals at one of these locations (Woody Isles) were then used to test our hypotheses that the mangrove corals relative to adjacent reef corals would (1) have enhanced respiration rates relative to net photosynthesis, (2) house different dominant Symbiodiniaceae taxa, and consequently (3) use different photosynthetic ‘strategies’ to survive the extreme physicochemical conditions. Through this work, we demonstrate that physiological plasticity (photosynthetic ‘strategy’) combined with unique symbiont diversity supports coral survival in the GBR mangrove lagoons, but that reductions in calcification rates are an associated survival trade-off. These findings contribute to the growing body of evidence that these traits are important for coral survival under suboptimal environmental conditions that are analogous to those predicted under climate change (Camp et al. 2018b).

2. MATERIALS AND METHODS

Five islands (Woody Isles, Two Isles, Hope Isles, Nymph Isles and Howick Island) adjacent to coral reefs on the GBR were investigated in June 2017 for prevalence of coral communities and associated abiotic conditions (Table 1). These sites were selected

based on local knowledge (J. Rumney pers. obs.) and prior knowledge of mangrove lagoon geomorphologies likely to house extreme coral communities (Camp et al. 2016a, 2017). Sites were initially examined via satellite imagery (Google Earth™) for key features (e.g. distance from a coral reef, presence of channels in the mangroves, their orientation to wind direction and image optical signatures characteristic of corals). Upon exploration, we found that 2 of these sites, Woody Isles (16.388° S, 145.566° E) and Howick Island (14.497° S, 144.972° E), housed corals living within the mangrove lagoons (Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m625p001_supp.pdf). Both sites had no visible significant freshwater catchment inputs and were semi-enclosed lagoons surrounded by mangrove forests. Coral community composition (cover, diversity and size-frequency distribution) and physicochemical conditions were determined, with additional seasonal abiotic sampling and assessment of coral physiology subsequently conducted at Woody Isles due to ease of boat access (February–April 2018, Table 1).

2.1. Environmental characterisation

Continuous sensor-based measurements (total pH [pH_T], salinity, temperature [°C] and oxygen [mg l⁻¹]) with SeapHOx™, combined with periodic spot-sampling (via discrete water samples analysed with a pH

Table 1. Summary of all research activities undertaken, including the date, site and habitat

Activity	Date	Site(s)	Habitat
Initial site exploration	June 2017	Woody Isles Howick Island Two Isles Hope Isles Nymph Isles	Mangrove lagoon
Benthic characterisation	June 2017	Woody Isles Howick Island	Mangrove lagoon & reef
High resolution (1 min) SeapHOx™ data collected at the mangrove lagoon over 48 h coupled with discrete water sample analysis from adjacent reef habitat (n = 12)	June 2017	Woody Isles Howick Island	Mangrove lagoon & reef
Seasonal (2 d in winter, n = 10; and 3 d in summer, n = 15) seawater carbonate chemistry analysis	June 2017 & February 2018	Woody Isles	Mangrove lagoon
Long-term (50 d) SeapHOx™ data	February–April 2018	Woody Isles	Mangrove lagoon
Rates of light and dark calcification, respiration and photosynthesis for <i>Acropora millepora</i> and <i>Porites lutea</i> (n = 4 per species and habitat)	April 2018	Woody Isles	Mangrove lagoon & reef
Fragment collection of <i>A. millepora</i> and <i>P. lutea</i> (n = 4 per species and habitat) for Symbiodiniaceae assessments	April 2018	Woody Isles	Mangrove lagoon & reef

meter [913, Metrohm] and multi-meter [3430, WTW] for temperature, salinity and oxygen) were used to characterise the physicochemical conditions at Woody Isles and Howick Island (see Table 1 for summary). Prior to sampling, all sensors were inter-calibrated for comparison consistency. Our initial site exploration in June 2017 only allowed for short (48 h) high-resolution (1 min sampling interval) data to be collected at the Woody Isles and Howick Island mangrove lagoons that were coupled with spot-sampling at the adjacent reef habitats (Table 1, Table S1). We used *t*-tests to compare data between habitats, with the SeapHOx™ data matching the time of spot-sampling used for the analysis.

At the Woody Isles mangrove lagoon, additional long-term deployment of the SeapHOx™ between February and April 2018 generated a 50 d (10 min resolution) dataset. The SeapHOx™ was deployed at ca. 1 m (at lowest tidal point), and discrete water samples were collected on initial deployment of the SeapHOx™ to corroborate the sensor readings (as per Camp et al. 2017). Linear regressions were conducted in SigmaPlot to assess how temperature, salinity and oxygen were influenced by tidal height and time of day (as per Camp et al. 2017). High tide and low tide data obtained from the Australian Bureau of Meteorology were matched to the corresponding SeapHOx™ data for that time to assess the effect of tidal height. Light (Lux) was measured through the deployment of HOBO® Pendant light loggers (Microdaq) and converted to photosynthetically active radiation (PAR, ca. 3% accuracy; see Long et al. 2012). Linear regressions were conducted from sunrise to daytime maximum (when light intensity is increasing, ca. 06:00–14:00 h) and then from daytime maximum through to the night-time (when light intensity is decreasing, ca. 14:10–05:50 h).

A seasonal comparison of the seawater carbonate chemistry was also undertaken at Woody Isles through the collection of discrete water samples (June 2017 and February 2018). Initial samples were collected at 07:00 h and repeated every 3 h until 19:00 h (2 d in winter, $n = 10$; and 3 d in summer, $n = 15$, Table 1). Seawater samples (500 ml) were stored in borosilicate bottles and fixed with HgCl_2 and sealed for later measurements of total alkalinity (A_T). Samples were transported back to the University of Technology Sydney where they were processed for A_T using an auto-titrator (916 TiTouch, Metrohm), verified with certified reference materials distributed by A. Dickson (Batch 137, Scripps Institute of Oceanography). Values of A_T were used with corresponding *in situ* pH_T , temperature, salinity and depth (m) to determine the remaining

carbonate system parameters, using CO2SYS, with the dissociation constants of Lueker et al. (2000) for K1 and K2, Dickson (1990) for KHSO_4 and Uppström (1974) for boric acid. Salinity ($S = 36$) dissolved inorganic carbon (nC_T) to total alkalinity (nA_T) plots were generated to assess the dominant mechanisms influencing the carbonate chemistry, whereby a location where calcification and dissolution are dominant processes has a linear regression slope approaching 2 (Suzuki & Kawahata 2003, Kleypas et al. 2011).

2.2. Benthic characterisation

To assess the coral cover and size-frequency distribution, continuous line intercept transects were undertaken in June 2017 (as per Gardner et al. 2019). At each location, 3 transects (30 m each) were randomly located and data recorded using a high definition camera (GoPro Hero5). Data were later analysed to the highest possible taxonomic resolution. Due to the heterogeneous nature of the mangrove lagoons, a coral species list was generated from all reported coral species observed during the sampling period.

2.3. Woody Isles coral physiology

Rates of light and dark calcification, respiration and photosynthesis were determined (April 2018) for 2 dominant coral species (*Acropora millepora* and *Porites lutea*) found within the Woody Isles mangroves and adjacent reef habitats ($n = 4$ per species per habitat, Table 1). Symbiodiniaceae associated with each coral species across habitats were also identified and quantified. Fragments (<5 cm) were collected from their native habitat and transported to the operations vessel ('Wavelength 5'), where they were incubated for 2 h in the light and then 2 h in the dark. Incubations were conducted in 250 ml glass chambers filled with native seawater that were continuously mixed via a magnetic stir plate. Prior to incubation, any non-live coral tissue (e.g. coral skeleton, attached rock/substrate) was covered with parafilm to prevent biological alteration of incubated seawater. Temperature was controlled via a water bath, and maintained within 0.2°C of the *in situ* ambient temperatures in the native environment (reef: 28.2°C , mangrove: 28.1°C). To determine an appropriate light intensity for incubation, we used the light saturation coefficient (E_k), which provides a measure of the long-term light history to which corals are acclimated (e.g. Suggett et al. 2012). Values of E_k were

determined using a pulse amplitude-modulated fluorometer (Diving PAM, Walz) and rapid light curves (RLCs) as detailed below, and were ca. 220 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ across all corals. Light intensity for the incubations was delivered via white-LED aquarium lights (Hydra, AI). Dark conditions were created by covering the chambers with black-out material.

Rates of light and dark calcification were determined by the alkalinity anomaly technique (Smith & Kinsey 1978) corrected for any changes in A_T of 3 seawater controls, during the light and dark incubations, respectively (as previously described by Camp et al. 2017). Net photosynthesis (P_N) and respiration (R) were determined by changes in oxygen during the light and dark incubations, respectively, corrected for changes in oxygen of the controls. Gross photosynthesis (G_p) was determined by the addition of P_N and R. All rates were normalised to the incubation volume, time and coral surface area, which was determined from 3D scans of the corals (Blue Light Geomagic capture) and the model software Geomagic Wrap. Additional fragments were taken from each coral species per habitat ($n = 4$) to determine the Symbiodiniaceae cell density. Tissue was removed from each nubbin with an air-pick, and an aliquot of the slurry was used to quantify cell counts using microscopy and normalised for surface area (Camp et al. 2016b). Two-way ANOVAs with post hoc Tukey HSD were conducted in R Studio version 1.1.423 (RStudio Team 2015) to compare habitat and species differences in physiological parameters. Tests of normality (Q-Q plots) and homogeneity of variance (Levene's test) were passed.

A diving PAM fluorometer with fibre optic was programmed (Measurement intensity [MI]: 12; Gain: 12; Saturating intensity [SI]: 12; Saturation width [SW]: 0.8s; Light curve interval [LC-INT]: 3) to deliver RLCs to assess for differences in photobiological traits of the coral-associated Symbiodiniaceae within each habitat (Suggett et al. 2012, Nitschke et al. 2018). For each RLC, acquisitions were applied with 8 actinic light steps and an initial dark measurement, whereby each light intensity was applied for 20 s. The actinic light levels (calibrated against a factory-calibrated LI-192 quantum sensor, Li-Cor) of the RLC were 0, 186, 214, 360, 456, 670, 1070, 1547 and 1975 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Minimum (F_o , F_o' , F') and maximum (F_m , F_m') fluorescence yields were obtained (where the prime notation denotes measurements performed in the light-acclimated state) and then used to calculate [1-C] and [1-Q] as per Suggett et al. (2015):

$$[1-C] = [(F_m' - F') / (F_m' - F_o')] \quad (1)$$

$$[1-Q] = [(F_m' - F_o') / F_m'] / [(F_m - F_o) / F_m] \quad (2)$$

Comparisons between [1-C] photochemical quenching (the fraction of open PSII reaction centres) versus [1-Q] dynamic nonphotochemical quenching was undertaken to assess differences in the photochemical quenching patterns of coral-associated Symbiodiniaceae within each habitat (Suggett et al. 2015, Nitschke et al. 2018).

2.4. Symbiodiniaceae diversity

Small fragments (1 cm) for each of *A. millepora* and *P. lutea* ($n = 4$) were removed from independent colonies and immediately preserved in RNAlater (Ambion, Life Technologies; Vega Thurber et al. 2009) and stored at -20°C until processing. Prior to DNA extraction, excess RNAlater solution was removed (Vega Thurber et al. 2009, Tout et al. 2015) before fragments were air-picked into sterile PBS-EDTA. DNA was extracted from the tissue slurry using the Qiagen DNeasy Plant Mini Kit according to the manufacturer's instructions. To disrupt the Symbiodiniaceae cells, 200 μl of 0.5 mm sterile glass beads (BioSpec) were bead-beaten at 30 Hz for 90 s with a Tissue Lyser II (Qiagen). Quantity and quality of DNA was checked using a NanoDrop 2000C spectrophotometer (Thermo Fisher Scientific). PCR amplifications were performed in triplicate reactions with a Qiagen Multiplex PCR Kit.

PCR amplification of the internal transcribed spacer 2 (ITS2) region was done using the primers ITSintfor2 and ITS2-reverse (Illumina sequencing adapters underlined), and following the PCR conditions of Arif et al. (2014):

ITSintfor2: 5'-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG GAA TTG CAG AAC TCC GTG-3'

ITS2-reverse: 5'-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGG GGA TCC ATA TGC TTA AGT TCA GCG GGT-3'

For individual PCR reactions, DNA was aliquoted to 12–50 ng l^{-1} , with 10 μl Qiagen Mix, 0.5 μl of each 10 M primer mix, 1 μl of DNA template, and DNase-free water to adjust the reaction volume to 20 μl . A 5 μl aliquot of each PCR product was run on an 1% agarose gel to visualise successful amplification. Samples were purified and indexed to add Nextera XT indexing and sequencing adapters (Illumina) according to the manufacturer's protocol. Indexed amplicons were again purified, quantified on the QuBit (Quant-IT dsDNA Broad Range Assay Kit; Invitrogen) and pooled in equimolar ratios on the BioAnalyser (Agilent Technologies). The final pooled library was purified on a 2% agarose gel to remove

excess primer-dimers. The library was sequenced at 8 pmol with 10% phiX on the Illumina Miseq, 2× 300 bp end version 3 chemistry according to the manufacturer's specifications, at the Ramaciotti Centre for Genomics.

The SymPortal analytical framework (Hume et al. 2019) was used to predict putative Symbiodiniaceae taxa (LaJeunesse et al. 2018) from the ITS2 marker. The framework uses next-generation amplicon sequencing data to identify specific sets of defining intragenomic ITS2 sequence variants (DIVs) that are the taxonomic unit of SymPortal (as the ITS2 type profile) indicative of genetically differentiated Symbiodiniaceae taxa. Demultiplexed and paired forward and reverse fastq.gz files outputted from the Illumina sequencing were submitted directly to SymPortal. Sequence quality control was conducted as part of the SymPortal pipeline using Mothur 1.39.5 (Schloss et al. 2009), the BLAST+ suite of executables (Camacho et al. 2009) and minimum entropy decomposition (Eren et al. 2015).

Differences in coral-associated Symbiodiniaceae between coral species and habitats were analysed using permutational multivariate analysis of variance (PERMANOVA). Fixed factors (species and habitat) were nested according to hierarchy, and 999 permutations of residuals were conducted based on Bray–Curtis distances between square-root transformed samples using the PRIMER-E software with the PERMANOVA+ add-on package v.1.0.6 (Clarke & Gorley 2006).

3. RESULTS

3.1. Environmental characterisation

The mangrove lagoons of Woody Isles and Howick Island had warmer temperatures and lower oxygen and pH conditions than the adjacent reef locations during the June 2017 assessment (Tables S1 & S2, *t*-tests, $p < 0.005$). As expected, both mangrove lagoons were characterised by large diel variations in temperature, pH and oxygen (Table 2). The long-term data collection at Woody Isles (February–April 2018; Table 1) revealed that repeatable diel variation influenced by both tide and time of day (Fig. 1). Tempera-

Table 2. Summary of Woody Isles and Howick Island mangrove lagoon physicochemical conditions. Data were collected via the SeapHOx™ as specified in Section 2.1 (and see Table 1). NA: data were not collected during this time point, and thus are not available

Physicochemical variable	Time	Site		
		Woody Isles	Howick Island	
pH (total scale)	Mean (SE)	February–	7.828 (0.001)	NA
	Range (CV)	April 2018	1.316 (0.031)	NA
	Mean (SE)	June 2017	7.871 (0.004)	7.847 (0.003)
	Range (CV)		0.488 (0.019)	0.640 (0.026)
Temperature (°C)	Mean (SE)	February–	28.3 (0.006)	NA
	Range (CV)	April 2018	7.7 (0.034)	NA
	Mean (SE)	June 2017	22.6 (0.008)	23.5 (0.014)
	Range (CV)		1.6 (0.015)	2.6 (0.024)
Oxygen (mg l ⁻¹)	Mean (SE)	February–	3.02 (0.007)	NA
	Range (CV)	April 2018	7.33 (0.366)	NA
	Mean (SE)	June 2017	3.91 (0.015)	4.14 (0.015)
	Range (CV)		3.19 (0.153)	3.40 (0.148)
Salinity	Mean (SE)	February–	32.5 (0.015)	NA
	Range (CV)	April 2018	16.5 (0.072)	NA
	Mean (SE)	June 2017	33.9 (0.013)	35.3 (0.018)
	Range (CV)		2.0 (0.016)	2.0 (0.020)

ture had greater influence than time of day, particularly during daylight hours ($R^2 = 0.303$, $n = 6628$, $p < 0.0001$), while pH_T and oxygen were influenced more by tidal height ($R^2 = 0.246$ and 0.210 respectively, $n = 238$, $p < 0.0001$; Fig. S2, Table S3).

Temperatures within the Woody Isles mangrove lagoon during the warmest months exceeded 33°C, while oxygen fell below 0.6 mg l⁻¹ and pH regularly dropped below pH 7.6, getting as low as pH 7.212 (Fig. 1). Salinity generally remained between 33 and 36; however, 3 large rainfall events (data obtained from the Australian Bureau of Meteorology) resulted in extreme reductions in salinity, with values decreasing to as low as 18 (Table 2, Fig. S3). Consequently, while the Woody Isles mangrove lagoon does not appear to have an inherent freshwater catchment, influxes of fresh water from the island during large storm events clearly occur. Daily PAR during the warmest months also revealed similar mean light intensities between habitats, with the mangrove lagoon having ca. 5% greater mean PAR than the reef habitat (Fig. S4).

Spot sampling conducted at the Woody Isles mangrove lagoon to assess the carbonate chemistry parameters (June 2017 and February 2018, Table 1) revealed relatively conserved aragonite saturation state (Ω_{arg}) across seasons (Table S4). The nA_T – nC_T slopes were 0.11 and 0.070 for the summer and winter, respectively, with minimal correlation between nA_T and nC_T ($R^2 < 0.02$; Fig. 2). Consequently, the

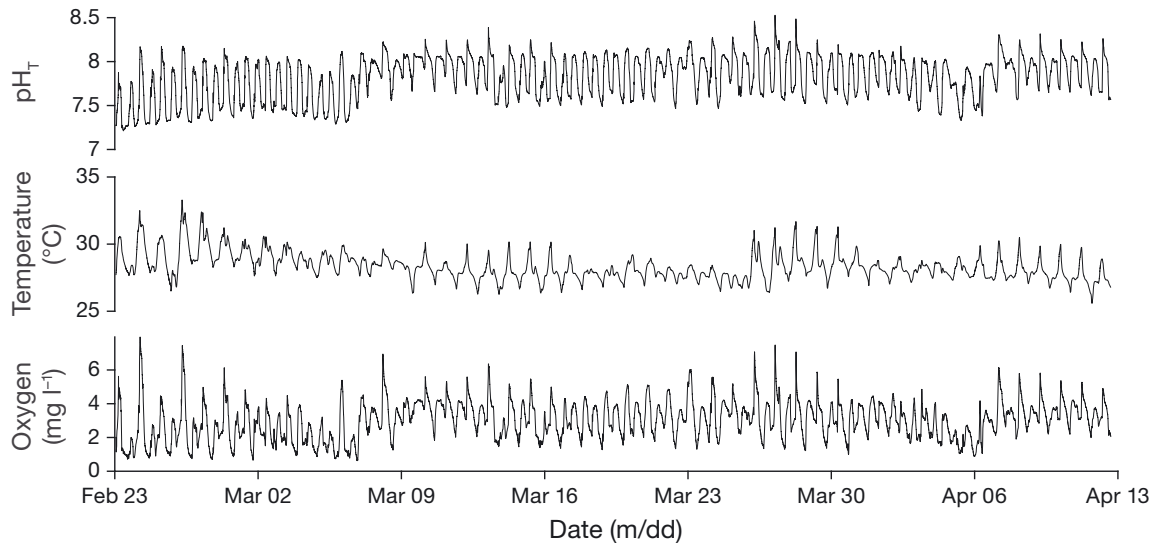


Fig. 1. Abiotic parameters (total pH [pH_T], temperature and dissolved oxygen) of Woody Isles mangrove lagoon on the Great Barrier Reef measured over a 50 d period between February and April 2018. Data were obtained from a SeapHO_xTM sensor set to log every 10 min

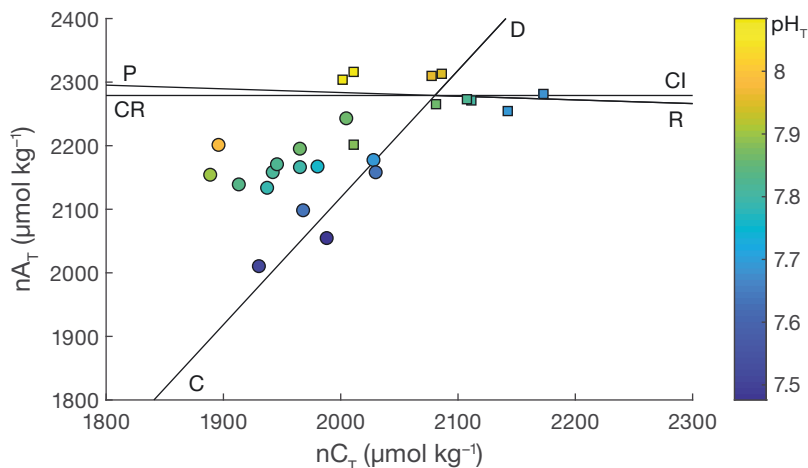


Fig. 2. Salinity-normalised ($S = 36$) total alkalinity (nA_T) and dissolved inorganic carbon (nC_T) plots for the Woody Isles mangrove lagoon during June 2017 (squares) and February 2018 (circles). Black lines represent the theoretical impact of calcification (C), carbonate sediment dissolution (D), photosynthesis (P), CO_2 release (CR), CO_2 invasion (CI) and respiration (R) on nA_T and nC_T . C and D are dominant processes when a linear regression slope approaches 2. Linear regression for each period was as follows: February 2018: $y = 0.11x + 1722.3$, $R^2 = 0.0217$; June 2017: $y = -0.07x + 2239.5$, $R^2 = 0.0018$

carbonate chemistry of the mangrove lagoon is not clearly dominated by calcification–dissolution or photosynthesis–respiration processes, highlighting the complex interplay of biogeochemical processes contributing to the abiotic conditions. However, a longer data series that also encapsulates storm events will ultimately be required to better resolve the dominant influences upon the mangrove lagoon complex carbonate chemistry.

3.2. Benthic characterisation

Despite the large variability in physicochemical conditions at the Woody Isles and Howick Island mangrove lagoons, both locations housed significant scleractinian coral communities. Coral cover at both mangrove lagoons was low (<5%, Fig. 3); however, broad visual exploration of the sites revealed that cover was highly heterogeneous, with some areas having much higher coral cover (see Figs. S1 & S5), while other areas lacked any corals. In total, 29 coral species were identified at the Howick Island mangrove lagoon, while 12 were identified at the Woody Isles mangrove lagoon (Table 3). Both sites housed corals with diverse growth forms (including: caespitose, corymbose, digitate, encrusting, foliose, massive and solitary) that were also taxonomically diverse. Both sites were

dominated by smaller colonies (<10 cm diameter), but Howick Island had larger coral colonies exceeding 0.5 m diameter (Fig. 3).

3.3. Woody Isles coral physiology

For 2 of the dominant coral species found within the Woody Isles mangroves and neighbouring reefs

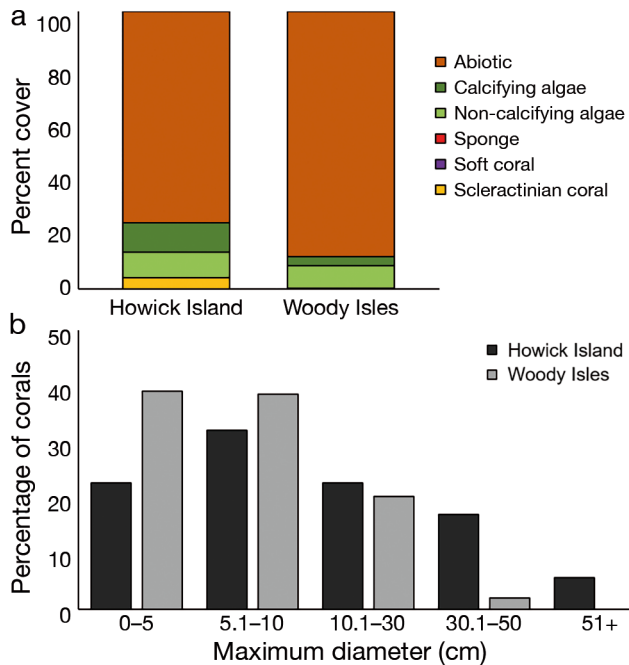


Fig. 3. (a) Percentage cover of major benthic taxa of the Howick Island and Woody Isles mangrove lagoons (see Fig. S5 for reef comparisons). (b) Size-frequency distribution of coral within the Woody Isles and Howick Island mangrove lagoons. Data are averaged from 30 m transects ($n = 3$) conducted within each habitat in June 2017

(*Acropora millepora* and *Porites lutea*), light calcification was reduced in the mangrove lagoon compared to the reef ($F_{1,12} = 4.96$, $p = 0.041$, reduction of 23% for *A. millepora* and 30% for *P. lutea*; Fig. 4, Table 4). Similarly, dark calcification was also reduced for corals in the mangrove lagoon relative to the reef ($F_{1,12} = 7.14$, $p = 0.017$; Fig. 4). For *P. lutea* from the mangrove lagoon, the reduction in dark calcification was comparable to the reduction in light calcification (ca. 22%), whereas for *A. millepora*, the reduction in dark calcification was 2 times greater than that of light calcification (ca. 47%; Fig. 4). Respiration was enhanced for both species in the mangrove lagoon ($F_{1,16} = 8.44$, $p = 0.01$), and while net photosynthesis was reduced ($F_{1,16} = 5.78$, $p = 0.029$; Table 4), compared to the reef, gross photosynthesis remained unchanged between habitats ($F_{1,16} = 0.004$, $p = 0.951$; Table 4).

Examination of Symbiodiniaceae cell density and genotype revealed distinct patterns for the corals across habitats. Symbiodiniaceae cell density was higher for colonies of both coral species in the mangrove lagoon than at the reef ($F_{1,12} = 1.5 \times 10^{31}$, $p < 0.0001$), which resulted in reduced gross photosynthesis per cell ($F_{1,12} = 203.7$, $p < 0.0001$; Table 4, Fig. S6). *P. lutea* experienced a mean (\pm SE) reduction in gross photosynthesis per cell from 1.60 ± 0.09 pmol

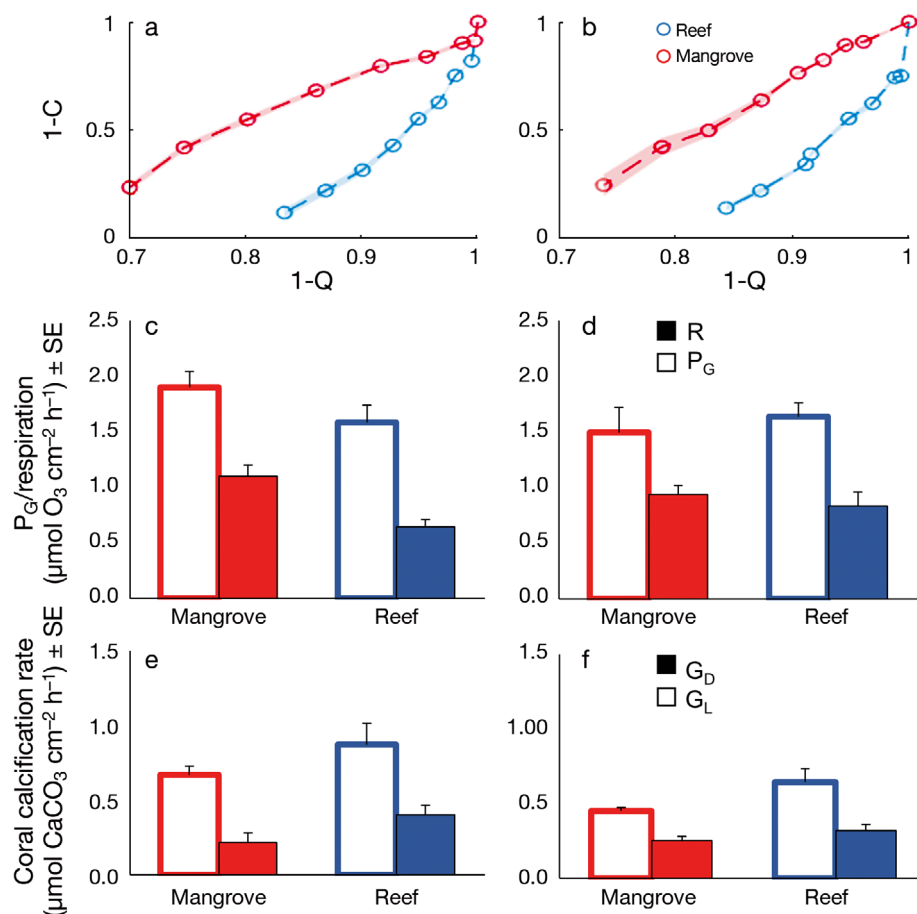
Table 3. Scleractinian coral species list for Woody Isles and Howick Island mangrove lagoons

Woody Isles	Howick Island
<i>Acropora millepora</i>	<i>Acropora cytherea</i>
<i>Acropora</i> sp.	<i>Acropora humilis</i>
<i>Acropora</i> cf. <i>nobilis</i>	<i>Acropora intermedia</i>
<i>Acropora</i> cf. <i>pulchra</i>	<i>Acropora tenuis</i>
<i>Acropora humilis</i>	<i>Acropora</i> cf. <i>valida</i>
<i>Astreopora gracilis</i>	<i>Astreopora gracilis</i>
<i>Coelastrea aspera</i>	<i>Coelastrea aspera</i>
<i>Favites</i> sp.	<i>Cyphastrea</i> sp.
<i>Montipora digitata</i>	<i>Goniastrea</i> sp. 1
<i>Pocillopora acuta</i>	<i>Goniastrea</i> sp. 2
<i>Porites cylindrica</i>	<i>Favia</i> sp. 1
<i>Porites lutea</i>	<i>Favia</i> sp. 2
	<i>Fungia fungites</i>
	<i>Lobophyllia corymbosa</i>
	<i>Lobophyllia</i> cf. <i>hemprichii</i>
	<i>Montipora digitata</i>
	<i>Montipora turtlensis</i>
	<i>Oulophyllia</i> sp.
	<i>Pavona</i> cf. <i>duerdeni</i>
	<i>Pavona</i> sp.
	<i>Platygyra sinensis</i>
	<i>Pocillopora acuta</i>
	<i>Pocillopora damicornis</i>
	<i>Porites cylindrica</i>
	<i>Porites lobata</i>
	<i>Porites lutea</i>
	<i>Seriatorpora</i> sp.
	<i>Turbinaria mesenterina</i>
	<i>Turbinaria reniformis</i>
Total $n = 12$	Total $n = 29$

O_2 cell⁻¹ h⁻¹ on the reef to 0.51 ± 0.03 pmol O_2 cell⁻¹ h⁻¹ in the mangrove lagoon, while *A. millepora* experienced a larger reduction from 5.40 ± 0.40 O_2 cell⁻¹ h⁻¹ on the reef to 0.52 ± 0.03 O_2 cell⁻¹ h⁻¹ in the mangrove lagoon (Fig. S6). This reduction of gross photosynthesis per cell across habitats was consistent with 'photosynthetic strategies' of the Symbiodiniaceae communities determined via the RLCs, which demonstrated 2 distinct habitat-specific excitation quenching patterns. Both coral species from the mangroves dissipated an increased proportion of absorbed excitation energy via dynamic nonphotochemical quenching ([1-Q]) rather than photochemical quenching ([1-C]) (Fig. 4a,b).

Genotypic characterisation of the associated Symbiodiniaceae taxa revealed a species and habitat effect (PERMANOVA; pseudo- $F_{1,13} = 9.82$, $p = 0.01$; Table S5). *A. millepora* associated with different Symbiodiniaceae genotypes between habitats, associating primarily with species of the genus *Cladocopium* on the reef (ITS2 type profile C3-C50c-C50a-C3b; although lesser abundances of *Durisdinium* species were detected), and *Durisdinium* in the

Fig. 4. Coral physiology of (a,c,e) *Acropora millepora* and (b,d,f) *Porites lutea* in the Woody Isles mangrove lagoon (red) compared to the neighbouring Woody Isles reef site (blue). Data were collected in April 2018 ($n = 4$ per species per habitat). (a,b) Photochemical quenching (1-C) versus nonphotochemical quenching (1-Q), (c,d) gross photosynthesis (P_G) and respiration (R), (e,f) light (G_L) and dark (G_D) calcification



mangrove lagoon (ITS2 type profile D2-D1ak-D1-D2.2) (Fig. 5). All *P. lutea* samples associated with C15 radiation (*Cladocopium*) taxa. At the mangrove lagoon, all samples associated with a single genotype (ITS2 type profile C15-C15by-C15bn), but at the reef, 3 distinct ITS2 type profiles (not including the mangrove ITS2 type profile) were identified in the 3 samples (C15, C15-C15bp-C15I-C15n-C15.8 and C15-C15I-C15n) (Fig. 5). As such, the changes in both photosynthetic capacity (gross photosynthesis, P_G) and strategy (greater non-photochemical dissipation) observed were paralleled by a change in Symbiodiniaceae community composition.

4. DISCUSSION

Identification of 2 mangrove lagoons on the Great Barrier Reef, housing diverse coral communities persisting under extreme abiotic conditions, contributes to a growing understanding of hot-spots supporting stress tolerant coral populations. As with previously identified mangrove lagoons housing corals (e.g.

Bouraké, New Caledonia; the Seychelles and Indonesia, see Table S6, Camp et al. 2016a, 2017) the abiotic conditions under which resident corals of Woody Isles and Howick Island are surviving frequently exceed Intergovernmental Panel on Climate Change (IPCC) predictions for seawater conditions in the year 2100 (IPCC 2014). Relative to other previously documented mangrove–coral habitats, the 2 GBR sites expose resident corals to frequently lower pH (e.g. below 7.3) and oxygen (e.g. below 1.0 mg l^{-1}) conditions, making them particularly extreme. The longer-term (50 d) Woody Isles physicochemical dataset revealed the dynamic nature of this specific mangrove lagoon, with a temperature range of ca. 7.7°C , and pH range of ca. 1.3 units (Table S6). In addition, the sporadic freshwater input from high rainfall events exposed resident corals to periods of highly variable salinity (range of 16.5 units). Such exposure to large environmental variance appears likely to influence species' responses to climate change (Rivest et al. 2017). For example, frequent exposure to more variable temperature often (Oliver & Palumbi 2011a, Palumbi et al. 2014, Rivest & Gouhier 2015, Schoepf

Table 4. Two-way ANOVA with post hoc Tukey HSD to assess significant interactions between coral species (*Acropora millepora* and *Porites lutea*) and habitat (mangrove lagoon and reef) for physiological parameters of corals around Woody Isles. *Indicates significant p-values ($p < 0.05$); NA: data were not collected during this time point, and thus were not available

Physiological parameter	Term	ANOVA			Post-hoc Tukey HSD significant interactions	
		F	df	p		
Net photosynthesis	Habitat	5.784	1,16	0.029*	NA	
	Species	5.425	1,16	0.034*		
	Habitat:Species	3.630	1,16	0.556		
Gross photosynthesis	Habitat	0.004	1,16	0.951	NA	
	Species	1.859	1,16	0.192		
	Habitat:Species	0.797	1,16	0.385		
Respiration	Habitat	8.444	1,16	0.010*	NA	
	Species	0.001	1,16	0.985		
	Habitat:Species	3.842	1,16	0.068		
Symbiodiniaceae density	Habitat	1.5×10^{31}	1,12	<0.0001*	Reef: <i>A. mil</i> -Mangroves: <i>A. mil</i>	p < 0.0001
	Species	5.0×10^{25}	1,12	<0.0001*	Mangroves: <i>P. lut</i> -Mangroves: <i>A. mil</i>	p < 0.0001
	Habitat:Species	1.2×10^{30}	1,12	<0.0001*	Reef: <i>P. lut</i> -Mangroves: <i>A. mil</i>	p < 0.0001
					Reef: <i>A. mil</i> -Mangroves: <i>P. lut</i>	p < 0.0001
					Reef: <i>P. lut</i> -Reef: <i>A. mil</i>	p < 0.0001
					Reef: <i>P. lut</i> -Mangroves: <i>P. lut</i>	p < 0.0001
Gross photosynthesis per cell	Habitat	203.730	1,12	<0.0001*	Reef: <i>A. mil</i> -Mangroves: <i>A. mil</i>	p < 0.0001
	Species	83.910	1,12	<0.0001*	Reef: <i>P. lut</i> -Mangroves: <i>A. mil</i>	p = 0.0112
	Habitat:Species	82.970	1,12	<0.0001*	Reef: <i>A. mil</i> -Mangroves: <i>P. lut</i>	p < 0.0001
					Reef: <i>P. lut</i> -Reef: <i>A. mil</i>	p < 0.0001
					Reef: <i>P. lut</i> -Mangroves: <i>P. lut</i>	p = 0.0104
Light calcification	Habitat	4.963	1,12	0.041*	NA	
	Species	6.393	1,12	0.022*		
	Habitat:Species	0.005	1,12	0.9430		
Dark calcification	Habitat	7.139	1,12	0.017*	NA	
	Species	0.247	1,12	0.626		
	Habitat:Species	1.453	1,12	0.246		

et al. 2015), but not always (e.g. Camp et al. 2016b), results in increased tolerance to thermal stress. However, how variance of multiple abiotic parameters influences stress tolerance remains poorly understood and requires further study, most critically since climate change will not only impact seawater temperature, but also other seawater properties, such as pH and dissolved oxygen (Mora et al. 2013). Despite the extreme physicochemical conditions, coral species found within the 2 GBR mangrove lagoons were taxonomically diverse, with species from the 2 major mitochondrially derived coral clades present (robust and complex; Romano & Palumbi 1996).

For 2 of the commonly occurring mangrove corals, *Acropora millepora* and *Porites lutea*, investigated further within this study, physiological plasticity, along with changes in associated Symbiodiniaceae taxa, appear important in facilitating coral persistence in the mangrove lagoon. Both of these traits ultimately influence energy acquisition, which becomes increasingly important as corals persist towards the edge of their fundamental niche (Sommer et al.

2014). Similar to our previous findings from New Caledonia (Camp et al. 2017), increased respiration rates were evident for coral species in the Woody Isles mangrove lagoon, accompanied by reductions in net photosynthesis and light and dark calcification rates (Fig. 4). It is likely that enhanced heterotrophic nutritional acquisition supports survival of the mangrove corals (see Camp et al. 2017), via provision of additional energy to support physiological maintenance, e.g. greater pH homeostasis at the site of calcification (Tambutté et al. 2011, 2015). *P. lutea* has also previously been documented to acclimate to high environmental variance in temperature and pH through adjustments of heterotrophy versus photosynthetic nutrition (Pacherres et al. 2013).

For both *A. millepora* and *P. lutea* in the Woody Isles mangrove lagoon, reductions in net photosynthesis were comparable to the reductions in light calcification (Fig. 4). Previous studies have also demonstrated a close coupling of calcification and photosynthesis (Gattuso et al. 1999, Langdon & Atkinson 2005, Anthony et al. 2008), although their exact relationship

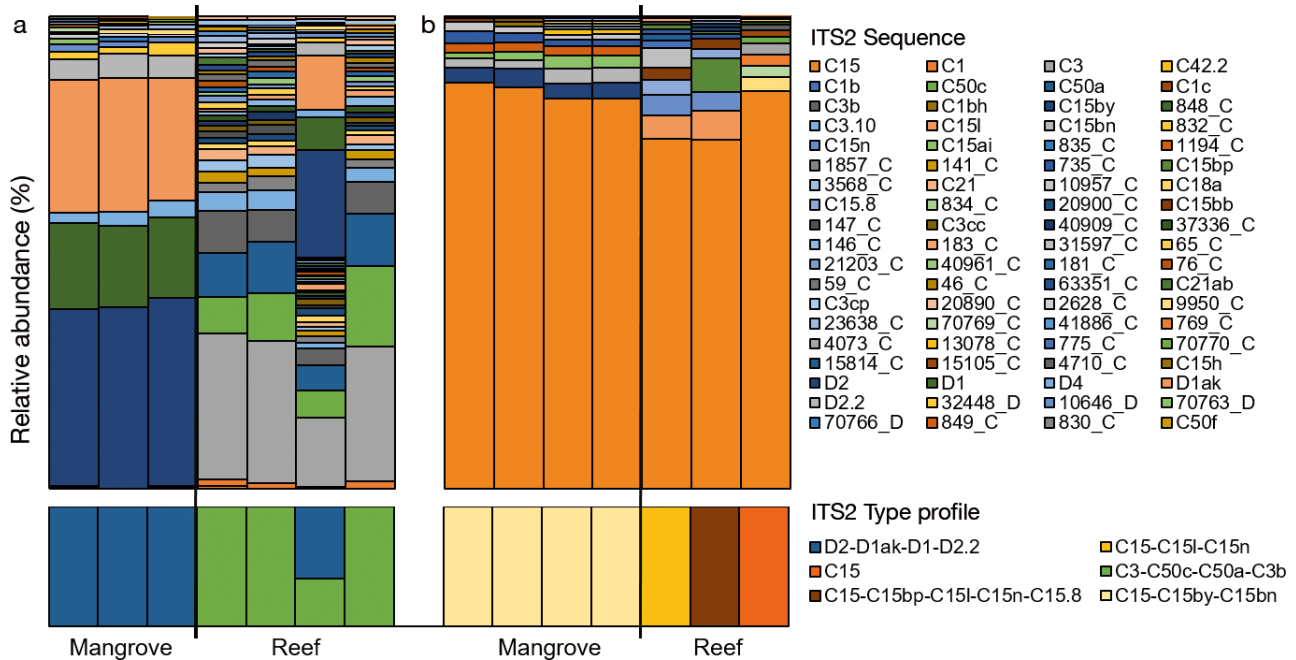


Fig. 5. Recovered ITS2 sequences and predicted ITS2 type profiles for (a) *Acropora millepora* and (b) *Porites lutea* across both the mangrove lagoon and reef habitats of Woody Isles on the Great Barrier Reef. Corresponding samples are plotted as stacked bar charts with a single column representing a sample. For each column in the stacked bar plots, the relative abundance of recovered ITS2 sequences is plotted in the upper section, while the relative abundance of predicted ITS2 type profiles is plotted below. Only ITS2 sequences contributing >0.01% in at least 1 sample are labelled. Sequences that have been designated names (e.g. C3, C15, or C3bm) refer to sequences that are commonly found in the literature or have been used to characterise ITS2 type profiles as part of this or previous analyses that have been run through the SymPortal analytical framework (Hume et al. 2019). Less common sequences, and those that have not been used to characterise ITS2 type profiles, are named according to a unique database ID and their clade/genera (e.g. 6417_C refers to a sequence with ID 6417 derived from a *Cladocopium* species, clade C)

remains unresolved at the polyp scale (Allemand et al. 2011, McCulloch et al. 2017). Recent evidence from *Porites* suggests that changes in Symbiodiniaceae metabolically derived dissolved inorganic carbon is more closely related to the pH of the corals' calcifying fluid, than the pH of the surrounding seawater (McCulloch et al. 2017). This suggests that the ability of corals to sustain photosynthesis could be crucial to their ability to calcify in extreme conditions, and consequently coral-associated Symbiodiniaceae are likely an important trait facilitating survival in extreme environments.

Both *A. millepora* and *P. lutea* exhibited a change in dominant Symbiodiniaceae taxa when persisting within the mangrove lagoon (Fig. 5). Both coral hosts also only associated with a species-specific single ITS2 type profile in the mangrove lagoon; for *A. millepora*, *Durusdinium* (ITS2 type profile D2-D1ak-D1-D2.2), and for *P. lutea*, *Cladocopium* (C15-C15by-C15bn), indicating specialised algal symbionts (Oliver & Palumbi 2011b), likely better adapted to the extreme conditions of the mangrove lagoon. Environmental conditions appear fundamental in influencing

optimal symbiotic partnerships (Putnam et al. 2012), with changing conditions altering the host's nutritional requirements (Suggett et al. 2017). We hypothesise that the resource landscape changes for corals when they persist into the extreme mangrove lagoons, and thus flexibility in associated Symbiodiniaceae can aid in meeting their metabolic requirements. For both coral species, the change in genotype was also accompanied by an increase in the number of Symbiodiniaceae cells and an overall reduction in gross photosynthesis per cell, with excitation energy dissipation preferentially directed into nonphotochemical quenching (Fig. 4). Symbiodiniaceae have different capacities to preferentially redirect electrons through nonphotochemical pathways—a trait that is known to be important under light stress, but is also likely to be important for exposure to other stressors such as pH and temperature (Suggett et al. 2015, Nitschke et al. 2018), which occur in the mangrove lagoon. Both coral hosts associated with symbionts in the mangrove lagoon that preferentially selected for nonphotochemical quenching over photochemical quenching (Fig. 4), suggest-

ing this is an important functional trait for host-symbiotic partners to have within the mangrove lagoon. However, association with a specialised Symbiodiniaceae may come at a cost to the host, such as reduced growth rates, as documented for associations with thermally tolerant *Durusdinium* symbiont species (Cunning et al. 2015).

Research into extreme inshore coral habitats, such as mangrove lagoons, is still in its infancy (Rivest et al. 2017, Camp et al. 2018b). Only with the increasing decline in global coral cover have researchers begun to consider the broader ecosystem value of such systems that includes preconditioning of corals to extreme conditions (Camp et al. 2016a). The heterogeneous nature of mangrove lagoons results in a variety of potential ecosystem services, including serving as a natural laboratory to study the multitude of complex stressors acting together to challenge the future survival of coral reefs (Camp et al. 2018b); refuge (Yates et al. 2014); climate change preconditioning (Camp et al. 2016a); and housing a potential stock of stress-tolerant corals (Morikawa & Palumbi 2019). For mangrove lagoons housing stress-tolerant corals, such as Woody Isles and Howick Island, further consideration should be given to the use of their native corals in active interventions, such as assisted gene flow (van Oppen et al. 2015, 2017), translocation of colonies to degraded areas (Anthony et al. 2017) and in coral nurseries (e.g. Suggett et al. 2019). The ability for these corals to tolerate multiple stressors suggests they would provide a valuable source of corals for reef intervention activities.

In consideration of the potential ecosystem services offered by mangrove lagoons, it is important to acknowledge that some sites, such as Woody Isles, only house isolated coral populations, compared to other locations, such as Howick Island, that house an accreting reef framework (Fig. S1). Also, the large reduction in species diversity found within these extreme systems relative to their adjacent reefs likely serves as a warning for future reefs as environmental stress intensifies. Further research is required to establish whether the reduction in species diversity results from a genetic or environmental bottleneck. Environmental filtering was postulated as one potential reason why some coral taxa were absent from a highly variable inshore habitat of the Cayman Islands (Lohr et al. 2017). Presence of both broadcast and spawning coral species suggests that reproductive mode does not explain the presence or absence of coral species from the mangrove lagoon habitats examined here. Individual species traits, water depth, available hard substrate for coral recruitment and

lagoon geomorphology appear important in contributing to the resident coral community composition. Further research is also urgently needed to understand if these extreme coral populations are already at the edge of their physiological capacity to cope with multiple stressors, ultimately how much additional stress they can survive under, and whether these extreme coral populations can maintain their stress tolerance when translocated to more environmentally stable reef habitats (Rivest et al. 2017). Despite these unknowns, it appears likely that these mangrove lagoon corals could be among the most capable to persist under future seawater changes over the coming century that will be comprised of a complex interaction of ocean warming, acidification and deoxygenation. To facilitate their survival, enhanced protection of these vital coral reef–mangrove interfaces is required, to limit additional stressors, e.g. nutrient or pollutant input that could damage these disproportionately valuable ecosystems that are still largely unexplored in the context of their role in supporting corals potentially adapted to future climates.

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