1 **Symbiont shuffling across environmental gradients aligns with changes in carbon uptake**
2 **and translocation in the reef-building coral *Pocillopora acuta***
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Abstract

Symbiosis between reef-building corals and unicellular algae (Symbiodiniaceae) fuels the growth and productivity of corals reefs. Capacity for Symbiodiniaceae to fix inorganic carbon (Ci) and translocate carbon compounds to the host is central to coral health, but how these processes change for corals thriving in environmental extremes remains largely unresolved. We investigate how a model coral – *Pocillopora acuta* – persists from a reef habitat into an adjacent extreme mangrove lagoon on the Great Barrier Reef. We combine respirometry and photophysiology measurements, Symbiodiniaceae genotyping, and ^{13}C labelling to compare *P. acuta* metabolic performance across habitats, in relation to the Ci uptake and translocation capacity by symbionts' autotrophy. We show that differences in *P. acuta* metabolic strategies across habitats align with a shift in dominant host-associated Symbiodiniaceae taxon, from *Cladocopium* in the reef to *Durusdinium* in the mangroves. This shift corresponded with a change in 'photosynthetic strategy', with *P. acuta* in the mangroves utilising absorbed light for photochemistry over non-photochemical quenching. Mangrove corals translocated similar proportions of carbon compared to the reefs, despite a lower Ci uptake. These trends indicate that coral survival in mangrove environments occurs through sustained translocation rate of organic compounds from coral symbionts to host.

36 **Introduction**

37 The ecological success of reef-building corals resides on their ability to establish and
38 maintain metabolic exchanges through an effective symbiotic association with dinoflagellates
39 from the family Symbiodiniaceae. Symbiodiniaceae fuel their hosts with organic carbon by
40 fixing inorganic carbon (Ci) through photosynthesis (Davy et al. 2012). While Ci uptake rates
41 by the algal symbionts have rarely been measured, they appear strongly regulated by
42 environmental factors, such as availability of CO_2 (pCO_2) (Suggett et al. 2012b; Brading et al.
43 2013) and temperature (Oakley et al. 2014). Recent work on cultured Symbiodiniaceae
44 revealed that different environmental optima primarily drive variation in Ci uptake rates (Ros
45 et al. 2020). Within reef systems where Symbiodiniaceae are hosted within cnidarian tissues,
46 symbiont cells are typically carbon-limited (Smith and Muscatine 1999; Doherty 2009;
47 Towanda and Thuesen 2012); as such, cnidarians can exhibit a stimulated carbon metabolism
48 under naturally higher pCO_2 (more acidic) environments (Suggett et al. 2012b). The efficiency
49 of Symbiodiniaceae carbon metabolism across environments thus appears an important trait in
50 supporting their host's survival, and a means to cope with stressful conditions.

51

52 Associations between the cnidarian host and specific genera, species or strains of
53 Symbiodiniaceae profoundly influence the stress resilience of their coral host (Berkelmans and
54 van Oppen 2006; Abrego et al. 2008; Howells et al. 2011; Oliver and Palumbi 2011). Corals
55 commonly host different Symbiodiniaceae across environmental gradients, presumably in
56 response to changes in available resources (Matthews et al. 2017; Suggett et al. 2017).
57 Increasing evidence from extreme coral environments are revealing host-specific changes in
58 associated Symbiodiniaceae that appear fundamental in supporting coral survival but suggest
59 that there is not a ubiquitous change across hosts when surviving in extremes. For example,
60 Hennige et al. (2010) observed a "shuffling" (*sensu* Baker 2003) of symbiont types from
61 *Cladocopium* to *Durusdinium* from optimal reef environments to mangrove waters for the coral
62 *Goniastrea aspera*. Conversely, Howells et al. (2016) found that *Platygyra daedalea* corals
63 shifted from *Durusdinium* in the mild temperature of the Sea of Oman to *Cladocopium* in the
64 hotter Persian-Arabian Gulf. Thus, host-Symbiodiniaceae plasticity appears central for corals
65 to acclimatise to a broad range of environmental conditions and expand their effective niche.

66

67 Whilst associating with different Symbiodiniaceae taxa appears to benefit corals
68 thriving into extreme environments (Berkelmans and van Oppen 2006; Howells et al. 2016;
69 Hume et al. 2016), these relationships are often associated with a 'trade off'. For example,

70 *Durusdinium* (type D1) symbionts translocate less photosynthetic compounds to their hosts
71 than *Cladocopium* (types C1 and C3; Cooper et al. 2011), and thus have been considered
72 somewhat "parasitic" (Lesser et al. 2013; Baker et al. 2018) or "selfish" (Stat and Gates 2011).
73 Under thermal stress, such "parasitism" can promote higher resource uptake but unchanged
74 resource translocation to the host for both *Symbiodinium* (type A3) and *Cladocopium* (type C7;
75 Baker et al. 2018). Consequently, host-Symbiodiniaceae associations that may promote stress
76 resilience might come with other metabolic costs that ultimately influence fitness, such as
77 reduced reproduction and calcification rates for corals associated with *Durusdinium* spp. (Jones
78 and Berkelmans 2011; Cunning et al. 2015). Understanding which host-Symbiodiniaceae
79 associations enable corals to persist under particularly suboptimal environments remains
80 largely unresolved, but is fundamental to forecast changes in corals metabolic performance and
81 productivity at the ecosystem scale (Camp et al. 2018).
82
83 Reef-building corals can thrive under unexpected suboptimal conditions, providing
84 unique opportunities to identify the physiological trade-offs underpinning their stress tolerance
85 (Palumbi et al. 2014; Camp et al. 2018; Burt et al. 2020). Such extreme environments include:
86 low light availability (mesophotic (Baird et al. 2018) or turbid (Suggett et al. 2012a; Sully and
87 van Woesik 2020)), warmer waters (e.g. Persian-Arabian Gulf; Ziegler et al. 2019), low salinity
88 (estuaries; Syahrir et al. 2018), or daily variation in multiple abiotic factors, such as
89 temperature, light, pH and O_2 (mangroves; Camp et al. 2019). Despite a combination of
90 stressors that reach on a daily basis the ranges predicted to occur on reefs by 2100 (Camp et al.
91 2016), reef-building corals are often abundant and healthy in mangrove systems (Camp et al.
92 2018). Coral colonies within and adjacent to mangroves can experience symbiont shuffling
93 (Hennige et al. 2010) and often exhibit up-regulation of the photosynthesis-to-respiration ratio
94 (P:R), mainly through enhanced respiration rather than photosynthesis (Hennige et al. 2010;
95 Camp et al. 2019). The processes involved in enhanced respiration in mangrove corals remain
96 unresolved, but could originate from increased host heterotrophy (feeding), increased
97 biological O_2 demand of associated bacteria (Gregg et al. 2013; Zhang et al. 2015), and/or
98 increased basal metabolism of the associated endosymbionts (Hill 2014).
99
100 Given the generally reduced net photosynthetic capacity and enhanced respiration
101 observed in corals adapted to mangrove lagoon environments (Camp et al. 2019), we tested the
102 hypothesis that this altered metabolism is due to reduced autotrophic carbon uptake and
103 translocation capacity by the algal symbionts. We sampled *Pocillopora acuta* coral colonies

104 from both the reef and neighbouring mangrove lagoon at Low Isles (Great Barrier Reef,
105 Australia). We first assessed coral metabolism using pulse-amplitude modulated (PAM)
106 fluorometry (energy quenching and photosynthetic potential), oxygen respirometry
107 (photosynthesis, respiration), as well as the symbiont ITS2 diversity. We then visualised and
108 quantified carbon uptake by Symbiodiniaceae, as well as translocation of photosynthates to the
109 coral host at the single cell level. Together, these approaches allowed us to compare carbon
110 uptake and translocation strategies of corals living in extreme and stable environments, to better
111 understand how these strategies could underpin coral survival in extreme mangrove lagoons.
112
113 **Materials and methods**
114
115 Collection of corals
116 Fifty coral fragments (< 5 cm) were sampled in May 2018 from 38 colonies of
117 *Pocillopora acuta* living at 1 m of depth (see detail on Table S1) at Woody Isles (16°23'10.3"S
118 145°33'53.9"E) mangrove lagoon and the adjacent Low Isles reef. Both sites have been
119 previously sampled and detailed as per Camp et al. (2019). Briefly, pH, temperature, dissolved
120 O_2, and salinity of the mangrove lagoon waters had more substantial diel variations than those
121 of the reef, and with overall lower pH and dissolved O_2, and warmer temperatures (see Table
122 S2), but similar range of light intensity. Light intensity recorded adjacent to the sampled corals
123 showed values at noon ranging from 455-576 µmol photons m^{-2} s^{-1} in the mangrove, with
124 parallel values of 442-483 µmol photons m^{-2} s^{-1} on the open reef (as detailed in Camp et al.
125 2019). Coral fragments were returned to the operations vessel (*Wavelength 5*) and processed
126 immediately. Corals were fragmented and left to recover in aerated aquaria for one hour in their
127 native seawater with frequent water changes to avoid temperature increases (target temperature
128 28.0 ± 0.2°C), with flow provided using aquarium pumps (2500 L/h). Respirometry and ^{13}C-
129 uptake incubations, as well as PAM fluorometry, were conducted on board the vessel, and
130 additional samples preserved for genomic analyses by flash freezing in liquid N_2 whilst on site.
131 Preserved samples were subsequently processed for further stable isotope and NanoSIMS
132 analyses back at the University of Technology Sydney, Australia.
133
134 Symbiodiniaceae photophysiology
135 Photophysiology measurements (n = 4) were made following low light acclimation (as
136 per Camp et al. (2019) using a PAM fluorometer (Diving PAM, Walz GmbH, Germany)
137 configured (MI: 12, Gain: 12, SI: 6 12, SW: 0.8s, LC-INT: 3) to collect rapid light curves

138 (RLCs) using eight actinic light sequences from 0 to 1975 µmol photons m^{-2} s^{-1}) of 20 seconds
139 duration. For each light sequence, minimum (F_o, F_o', F'; where F_o' is the minimum PSII
140 fluorescence yield calculated as Oxborough and Baker (1997) and F' is the fluorescence yield
141 under actinic light) and maximum (F_m, F_m') fluorescence yields (where the prime annotation
142 represents measurements performed in the light-acclimated state; instrument units) were
143 recorded. These parameters were then used to calculate the maximum yield of photosystem II
144 (PSII) photochemistry (F_v/F_m, where $F_v = F_m - F_o$; dimensionless), the photochemical and
145 dynamic non-photochemical quenching ([1-C] and [1-Q], respectively; dimensionless; see
146 Eqns. 1 and 2) as per Suggett et al. (2015):
147
148 $[1 - C] = ((F_m' - F')/(F_m' - F_o'))$ Eqn. 1
149 $[1 - Q] = ([F_m' - F_o']/F_m')/(F_v/F_m)$ Eqn. 2
150
151 Where photochemical quenching [1 - C] describes the use of absorbed excitation energy for
152 photosynthesis and non-photochemical quenching [1 - Q] represents absorbed excitation
153 energy emitted as heat and therefore not delivered to the photosynthetic electron transport
154 chain.
155
156 Coral holobiont photosynthesis and respiration rates
157 Coral nubbins collected from both the mangrove lagoon and reef sites (n = 5 per habitat)
158 were incubated for 2 hr (in the light and dark, respectively) in gas tight sealed 250 mL glass
159 incubation chambers continuously stirred using a magnetic stirrer, as per Camp et al. (2019).
160 Prior to incubation, any non-live coral tissue was covered with Parafilm to minimise other
161 biological alterations influencing the seawater. Each chamber was filled with seawater from
162 the source habitat and placed in a water bath maintained at 28.0 ± 0.2°C, matching discrete
163 measurements of seawater temperature for the same day and time of collection (reef: 28.2°C
164 and mangrove: 28.1°C) as measured using a 3430 multi-meter (WTW GmBH, Germany). The
165 water bath was maintained under artificial white-LED light intensity of 350-400 µmol photons
166 m^{-2} s^{-1} (Hydra Fifty Two HD LED, AquaIllumination, Ames, IA, USA), verified using a 4π LI-
167 190SA Quantum Sensor (LI-COR, Lincoln, NE, USA). Rates of light and dark respiration (R),
168 net photosynthesis (P_N) and subsequently gross photosynthesis (P_G, where $P_G = P_N + R$) were
169 determined by measuring O_2 values at the beginning and the end of the incubation with an O_2
170 probe connected to a FireStingO_2 oxygen meter (PyroScience GmbH, Germany). A two-point

171 calibration of the optode was performed using both 0% air saturated seawater (achieved by
172 addition of sodium sulphite) and 100% air saturated seawater following manufacturer
173 guidelines. Control incubations were performed using native seawater without corals (Camp et
174 al. 2019) and any measured changes in O_2 were subtracted from rates of P_G or R accordingly.
175 Rates of O_2 production and consumption were normalised by incubation volume, time, and
176 either Symbiodiniaceae cell density or coral skeleton surface area. The selected light intensity
177 and incubation length represented a balance between providing light-saturation for O_2
178 evolution and constraining large drift in pO_2 over the incubation that could otherwise induce
179 pO_2-dependent variability in P_G:R and/or expose corals to hyperoxia or hypoxia stress (e.g.
180 Hughes et al. 2020).
181
182 <u>^{13}C isotope labelling and enrichment analysis</u>
183 Inorganic carbon uptake incubations were performed on two different fractions of the
184 corals: freshly isolated symbionts (FIS) (See Supplementary Information) and intact coral
185 fragments (holobiont). Both fractions were from different branches of the same colony for both
186 mangrove lagoon and reef environments, and for 5-7 colony replicates. Both fractions were
187 then incubated for 3 hours within gas-tight Parafilm-sealed 400 mL glass incubation chambers
188 with autoclaved artificial seawater (ASW, Berges et al. 2001) at pH 8.02, with a final
189 concentration of 2 mM $NaH^{13}CO_3$ (^{13}C isotopic abundance of 98%, Sigma-Aldrich), and
190 attached with carabineers to a metallic grid fitted at the bottom of the same water bath setup as
191 for respirometry incubations, but with a white-LED light intensity of 700-800 µmol photons
192 m^{-2} s^{-1}. Light and temperature were monitored as per respiratory incubations and recorded at 5
193 min intervals for the duration of the experiment by HOBO Pendant data loggers (Onset, MA,
194 USA).
195
196 *Intact host-Symbiodiniaceae fraction*s: Before incubation, small subsample fragments (1 cm)
197 of each replicate coral fragment were flash frozen in liquid N_2 to assess the natural carbon
198 isotope ratio. Corresponding fragments (4 cm) were incubated as per the FIS (above), and upon
199 termination of incubation, were immediately rinsed with non-labelled ASW. For each replicate,
200 one additional fragment (1 cm) was subsampled post-incubation and kept for chemical fixation
201 before NanoSIMS analysis. The remaining fragment was flash frozen in liquid N_2 for later
202 enrichment analysis. Upon return to the laboratory, all frozen coral fragments were thawed,
203 airbrushed in filtered ultrapure water, and symbionts were separated from the host fraction at

204 3,000 RCF for 5 min (Treignier et al. 2008). Both host and symbiont fractions were treated
205 separately for stable isotope analysis.
206
207 Samples for all three fractions generated from the incubation experiment (FIS incubated
208 *ex hospite*, symbionts *in hospite* and host coral) as well as their corresponding controls sampled
209 before incubation were resuspended and rinsed after three extra steps of centrifugation-
210 resuspension in ultrapure water to remove residual salts interfering with the elemental analysis.
211 Suspensions were placed in acid-washed, pre-combusted borosilicate vials, flash frozen in
212 liquid N_2 and freeze-dried (Alpha 2–4 LDplus, Martin Christ GmbH, Germany) for 48 hrs prior
213 to encapsulation according to UC Davis Stable Isotope Facility (Davis, CA, USA) guidelines.
214 Enrichment analysis was performed with a PDZ Europa ANCA-GSL elemental analyser
215 interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (EA-IMRS, Sercon Ltd.,
216 Cheshire, UK). Enrichment levels were normalised relative to the natural isotope abundance in
217 unlabelled samples and expressed using $\delta^{13}C$ notation. Enrichment of $\delta^{13}C$ (expressed in ‰)
218 was quantified as follows:

219 $$\delta^{13}C = \left(\left(\frac{C_{sample}}{C_{unlabelled}}\right) - 1\right) \times 10^3$$

220 Where C is the $^{13}C/^{12}C$ atom ratio of the incubated sample or unlabelled control, respectively.
221
222 <u>NanoSIMS preparation and analysis</u>
223 NanoSIMS analysis was performed on both freshly extracted symbionts and holobionts
224 to visualise and confirm cellular Ci uptake and, in the case of the holobiont fraction,
225 translocation of labelled compounds from the symbionts to their host. Labelled holobiont
226 fragments (1 cm) and aliquots (250 µL) of the symbiont extracts were chemically fixed on the
227 research vessel following the methods of Pernice et al. (2014) for 24 hours at 4°C in a solution
228 containing 1% formaldehyde and 2.5% in PBS-sucrose buffer (0.1 M phosphate, 0.65 M
229 sucrose, and 2.5 mM $CaCl_2$), pH 7.5. After fixation, samples were rinsed and stored in PBS-
230 sucrose buffer at 4°C. Holobiont fragments underwent an extra step of decalcification process
231 at 4°C and pH 7.5 using PBS-EDTA (0.1 M phosphate, 0.5 M EDTA) changed every 24 hours,
232 until complete dissolution of the skeleton, and then stored in PBS buffer (0.1 M phosphate, pH
233 7.5) at 4°C until further processing (Pernice et al. 2014). Two coral polyps were dissected from
234 the coral tissue of the fragment and fixed in 1% OsO_4 - PBS (0.1 M phosphate, pH 7.5).
235 Dissected polyps and FIS were then dehydrated in increasing gradients of ethanol (50%, 70%,
236 90% and 100%) followed by acetone (100%) and infiltrated with incremental gradients of

237 SPURR resin (25%, 50%, 75%, and 100%) before embedding in moulds and polymerisation
238 of the resin at 65ºC. Ultrathin sections of 200 nm were then cut using a diamond blade,
239 deposited on silicon wafers, then gold-coated (10 nm) for NanoSIMS analysis.

240 Sections were analysed on a NanoSIMS-50 (Cameca, Gennevilliers, France) at the
241 Centre for Microscopy, Characterisation and Analysis at the University of Western Australia.
242 Five isotopic species were simultaneously collected ($^{12}C_2^-$, $^{12}C^{13}C^-$, $^{12}C^{14}N^-$, $^{12}C^{15}N^-$, and ^{32}S).
243 Enrichment of ^{13}C was confirmed by an increase in the carbon ($^{13}C/^{12}C$) ratio above natural
244 abundance values recorded in controls (0.011). Samples of interest were rastered with a ~ 2.5
245 pA Cs^+ beam (~100 nm diameter) across 50 μm^2 areas (512 × 512 pixels), with a dwell time of
246 15 ms per pixel. Data were simultaneously collected for $^{12}C^{12}C^-$, $^{13}C^{12}C^-$, and $^{12}C^{14}N^-$secondary
247 molecular ions with mass resolving power (MRP, Cameca definition) > 8000 (sufficient to
248 separate $^{13}C^{12}C^-$ from $^{12}C_2{}^1H^-$). NanoSIMS data were processed and analysed using Fiji
249 (Schindelin et al. 2012; http://fiji.sc/Fiji) with the Open-MIMS plug-in
250 (https://github.com/BWHCNI/OpenMIMS). Images were corrected for detector dead time (44
251 ns) on individual pixels before $^{13}C^{12}C^-/^{12}C^{2-}$ ratio images were generated using a colour-coded
252 transform (hue saturation intensity, HSI) showing natural abundance levels in blue, and
253 increasing enrichment of ^{13}C represented by the shift in colour towards magenta (set to
254 represent maximum enrichment). Regions of interest (ROIs) were manually selected to
255 represent key features (symbiont cells, FIS and *in hospite*; and gastrodermal host tissue
256 surrounding endosymbionts) and total ion counts calculated for each and used to generate ^{13}C
257 enrichment of each ($^{13}C^{12}C^-/^{12}C^{2-}$). Ratios were calibrated by taking daily measurements of a
258 *Saccharomyces cerevisiae* standard independently analysed by IRMS (corrected against
259 Vienna-Pee-Dee Belemnite).
260
261 Symbiodiniaceae cell density and skeletal surface area

262 Aliquots of 50 μL of FIS stored in ASW were counted on the same day of extraction
263 using a Neubauer chamber and a compound light microscope. Triplicate counting of each
264 sample was made to reach a minimum of 200 cells per chamber and then averaged to obtain
265 the cell concentration. The skeletal surface areas of airbrushed branches were estimated using
266 the wax weight method following Stimson and Kinzie (1991). Eight metallic objects of known
267 surface area (10.3 – 60.9 cm^2) were wax-coated in the same way as corals skeletons to produce
268 a standard curve of wax weight per surface area ($y = 0.0405x – 0.1051$; $R^2 = 0.9957$). Measured
269 surface areas of the broken parts of the coral skeletons were then subtracted from the value
270 obtained with the standard curve to account for areas where Symbiodiniaceae were not present.

271

272 Symbiodiniaceae ITS2 identity

273 Fragments of 1 cm were taken from independent colonies of *P. acuta* at each site
274 (n = 4), preserved in RNAlater (Ambion, Life Technologies, Australia) and stored at -20°C
275 until further processing. Excess RNAlater solution was removed prior to DNA extraction (Tout
276 et al. 2015). Fragments were then airbrushed with sterile PBS-EDTA (0.1 M phosphate, 0.5 M
277 EDTA) into small sterile zip lock bags until a slurry of coral tissue was formed. Extraction of
278 the DNA from the slurry was performed using the Qiagen DNeasy Plant Mini Kit (Qiagen,
279 Hilden, Germany) and cells disrupted with a Tissue Lyser II (Qiagen, Hilden, Germany) as per
280 Camp et al. (2019). The quantity and quality of the extracted DNA were checked using a
281 NanoDrop 2000C spectrophotometer (Thermo Fisher Scientific, MA, USA). For PCR
282 reactions, amplification of the ITS2 region was performed using ITS2-reverse and ITSintfor2
283 primer pairs (Camp et al. 2020), following previously published PCR conditions (Arif et al.
284 2014). Amplicons were sequenced using the Illumina Miseq platform (2 x 300bp) at the
285 Ramaciotti Centre for Genomics (University of New South Wales, Australia). Output files of
286 the Illumina sequencing were then submitted to the SymPortal analytical framework (Hume et
287 al. 2019) and quality controlled using Mothur 1.39.5 (Schloss et al. 2009), minimum entropy
288 decomposition (Eren et al. 2015), and BLAST+ executable suites (Camacho et al. 2009) to
289 predict Symbiodiniaceae taxa (LaJeunesse et al. 2018) from the ITS2 marker. All raw sequence
290 data are accessible under NCBI's BioProject (PRJNA630092).

291

292 Coral Host identity

293 Extraction of the coral DNA was performed on the coral holobiont slurry using a
294 phenol-chloroform-isoamyl alcohol protocol (see Supplementary Information) modified from
295 Guthrie et al. (Guthrie et al. 2000). Extracted DNA was amplified by PCR towards the
296 mitochondrial open reading frame (ORF) region (Flot and Tillier 2007) using host-specific
297 forward Pdam-F and reverse Pdam-R primers (Torda et al. 2013). PCR cycle conditions were
298 as per Torda et al. (2013). PCR amplicons were then Sanger-sequenced at the Australian
299 Genomic Research Facility, NSW. Sequences were aligned in Geneious v.6.0.6 against
300 reference sequences for *Pocillopora* from NCBI and matched with reference sequences for *P.*
301 *acuta* (Schmidt-Roach et al. 2014) and confirmed initial identification based on morphology.

302

303 Statistical analyses

304 Statistical analyses on physiological data were performed using SPSS Statistics 25
305 (IBM, Armonk, NY, USA). To assess the differences in photosynthetic strategies between
306 corals from the reef and the mangrove, the slopes of the linear trends (y = ax + b) fitted for
307 each technical replicate on the [1 – C] versus [1 – Q] curves were compared. Data series were
308 tested for normality (Shapiro-Wilk test) and homoscedasticity (Levene's homogeneity test).
309 Upon confirmation of normality and homoscedasticity, independent sample one-tailed t-tests
310 were used to compare differences between mangrove and reef (respirometry, cell density and
311 photosynthetic strategy). Differences in Ci uptake between each site across all sample fractions,
312 and between all sample fractions across each site were assessed with One-way ANOVAs with
313 post-hoc Tukey HSD to determine between which samples fractions the differences occurred
314 (elemental analysis) and with Mann-Whitney U tests (NanoSIMS). Significant outliers were
315 removed using the Grubb's test for outliers. For all statistical tests, alpha (α) was set to 0.05.
316
317 **Results**
318 <u>Reef and mangrove coral physiology</u>
319 Respiration (R) and gross photosynthesis (P_G) rates were similar for *P. acuta* from the
320 reef and the mangrove (Figure 1a). Both R and P_G normalised to coral surface area exhibited
321 no difference between mangrove and reef corals (R: 0.27 ± 0.09 on the reef and 0.16 ± 0.02
322 µmol O_2 cm^{-2} h^{-1} in the mangrove; $t_{(8)} = 1.19$, $P = 0.134$ and P_G: 0.67 ± 0.14 on the reef and
323 0.56 ± 0.07 µmol O_2 cm^{-2} h^{-1} in the mangrove; $t_{(8)} = 0.68$, $P = 0.258$). Symbiodiniaceae cell
324 density per surface area (see Figure S1) was significantly higher ($t_{(10)} = 5.02$, $P < 0.001$) for
325 mangrove corals ($1.31 \times 10^6 \pm 4.87 \times 10^4$ cells) compared to the reef ($6.88 \times 10^5 \pm 9.79 \times 10^4$
326 cells). Consequently, values of P_G when normalised per symbiont cell (P_G $cell^{-1}$; Figure 1b)
327 were ultimately 37.80% lower ($t_{(8)} = 2.06$, $P = 0.037$) for corals in the mangrove lagoon (0.51
328 ± 0.11 pmol O_2 $cell^{-1}$ h^{-1}) compared to the reef (0.82 ± 0.10 pmol O_2 $cell^{-1}$ h^{-1}). Analysis of PSII
329 photochemical quenching patterns (Figure 1c) demonstrated that *P. acuta* preferentially
330 utilised absorbed excitation energy through photochemical quenching ([1 - C], PSII
331 photosynthesis) rather than dissipating as heat via non-photochemical quenching ([1 - Q]
332 photoprotection) for samples from the mangrove compared to the reef ($t_{(8)} = 3.09$, $P = 0.007$).
333
334 Patterns of ^{13}C uptake by Symbiodiniaceae and translocation to their host at the bulk
335 scale (Figure 2a), followed the trends observed for P_G $cell^{-1}$ for *P. acuta* from the two habitats.
336 Symbionts living *in hospite* in the mangrove corals fixed ($F_{1.8} = 8.52$, $P = 0.019$) and
337 translocated ($F_{1.8} = 12.65$, $P = 0.007$) 29.88% less carbon than those *in hospite* in the reef

338 corals. However, the proportion of translocated carbon relative to total ^{13}C uptake was the same
339 for corals from both habitats (ca. 22% of total fixed carbon was in the host fraction). ^{13}C uptake
340 by FIS was the same for samples from both habitats ($F_{1,8} = 1.082$, $P = 0.329$), but approximately
341 91% less than for the Symbiodiniaceae when *in hospite* (see Supplementary Information).
342
343 ^{13}C uptake at the single cell level revealed that Ci uptake was generally 2.65 times
344 greater ($U = 7$, $P < 0.01$) *in hospite* (Figure 2) for samples from the reef compared to the
345 mangrove. Additionally, FIS followed the same trend (see Figure S2). Both holobiont fractions
346 exhibited enriched areas around the symbiont cells, corresponding to host storage bodies
347 (Figure 2c-f), with no significantly different $\delta^{13}C$ in enriched areas (~ 344 ‰) between reef
348 and mangrove corals (Figure 2b).
349
350 Symbiont identities from mangrove and reef corals
351 Differences in photobiology, P_G and Ci uptake (and translocation) for reef versus
352 mangrove were consistent with a shift in dominant Symbiodiniaceae taxa within *P. acuta*
353 across habitats. No major ITS2 type profiles were shared between corals from the mangrove or
354 reef habitats (Figure 3). Colonies of *P. acuta* associated with Symbiodiniaceae from the genus
355 *Cladocopium* on the reef, while in the mangrove the association was mostly with the genus
356 *Durusdinium*. Three major ITS2 types (proxies for Symbiodiniaceae genotypes) were observed
357 in the reef colonies, with C1/C1b and C1d-C42.2 (present in three replicates), and C1k-C3cg
358 (present in one replicate). In the mangrove corals, the major ITS2 type profile belonged to
359 D6/D1-D4-D2.2, with a replicate also harbouring D1h type exclusively. Notably, no ITS2
360 sequence belonging to *Durusdinium* was identified in reef corals and only four ITS2 sequences
361 belonging to *Cladocopium* (C15, C1, C1d and C3) were shared between the reef and mangrove
362 corals. These *Cladocopium* represented less than 0.02% cumulatively in three of the four
363 mangrove replicates.
364
365 **Discussion**
366 Accelerating degradation and loss of coral reefs worldwide due to anthropogenic
367 impacts have created an urgent need to identify coral populations with greater natural resilience
368 to stress (Hoegh-Guldberg et al. 2018). Corals that already persist in present-day environmental
369 extremes present important model organisms to identify mechanisms that support stress-
370 resilience (Camp et al. 2018). Here, we contrasted corals inhabiting extreme mangrove habitats
371 and adjacent reefs to identify the mechanisms enabling them to survive the warmer

372 temperatures, low pH and low O_2 conditions, analogous to future climate predictions. By
373 assessing photosynthetic and respiratory rates, light-dependent dynamic quenching patterns
374 and direct inorganic carbon (Ci) uptake by the symbiont and translocation to the host, we show
375 that the coral *P. acuta* likely employs metabolic strategies that differ between habitats, however
376 also reflects differences in symbiont density and types. In the mangrove lagoon, *P. acuta*
377 endosymbionts appear more efficient at utilising absorbed light for photochemistry but are less
378 able to fix Ci (or translocate fewer photosynthates to their host), confirmed by both stable
379 isotope analysis and NanoSIMS imaging.
380
381 Symbiont shuffling across environmental gradients
382 *P. acuta* associated with Symbiodiniaceae from the genus *Durusdinium* in the
383 mangrove lagoons but *Cladocopium* on the reef. Previous work from Low Isles (Camp et al.
384 2019) also demonstrated a shift of *Cladocopium* to *Durusdinium* for *Acropora millepora*
385 between mangrove and reef. In congruence with our findings, switching from *Cladocopium* to
386 *Durusdinium* symbionts has been commonly observed when environmental conditions become
387 suboptimal (Hennige et al. 2010; Boulotte et al. 2016; Cunning et al. 2018). However, corals
388 can persist in suboptimal environments in association with other Symbiodiniaceae genera, e.g.
389 the back-reef pools of American Samoa (*Cladocopium*; Barshis et al. 2010), and the mangroves
390 of New Caledonia (*Symbiodinium* and *Cladocopium*; Camp et al. 2020) and Australia
391 (*Cladocopium*; Camp et al. 2019)). Our findings support the body of evidence that shuffling of
392 symbionts to a preferential association within a given environment is host-specific. Host-
393 symbiont shuffling could be a long-term adaptation to stressful conditions, with evidence from
394 the coral *Montipora digitata* showing that shuffled symbionts tend to persist over multiple
395 generations (Quigley et al. 2019).
396
397 Reliance of mangrove *P. acuta* on photochemical quenching
398 Light-dependent dynamic quenching assessment is a diagnostic tool to assess
399 photosynthetic "strategy" (Suggett et al. 2015; Nitschke et al. 2018) and the preference of cells
400 to direct absorbed excitation energy towards photochemical ([1 - C]) or non-photochemical
401 pathways ([1 - Q]) (Kanazawa and Kramer 2002). A previous study (Camp et al. 2019) also
402 investigated physiological trade-offs associated with the survival in the same mangrove lagoon
403 of two other coral species (*A. millepora* and *Porites lutea*). We see a convergence of strategies
404 for all three species of a reduction in P_G and R (normalised to cell density present *in hospite*)
405 and P_G:R from reef to mangrove (Figure 4a) that appears mainly driven by the large increase

406 in cell density of the mangrove corals. A decrease in P_G:R for all three coral species from reef
407 to mangrove may be indicative of preferential reliance on heterotrophy rather than autotrophy
408 (Goyen et al. 2019) for mangrove corals; however further studies investigating the plasticity of
409 this ratio over a diel cycle (as well as actual measures of heterotrophy) would be required to
410 assert trophic strategies between reef and mangrove corals. Moreover, the extent of variation
411 was different between coral species, with a higher shift for *A. millepora* compared to *P. acuta*.
412 Absorbed excitation energy was preferentially dissipated via [1 - Q] for *A. millepora* but by [1
413 - C] for *P. acuta* for mangrove compared to reef populations (Figure S3), despite the fact that
414 light intensity is generally similar across the two habitats (Camp et al. 2019). The reduced
415 reliance on photochemical quenching is consistent with reduced P_G $cell^{-1}$ for *A. millepora*.
416 However, for mangrove *P. acuta*, preferential reliance on [1 - C] was accompanied by a reduced
417 P_G $cell^{-1}$ – indicating a higher capacity to process absorbed electrons that does not lead to higher
418 net O_2 release. This phenomenon could be explained by a relatively higher proportion of "light-
419 dependent O_2 consumption" through processes such as photorespiration and chlororespiration
420 that can serve as photoprotective electron sinks to dissipate excess excitation energy (Hughes
421 et al. 2018). Such a photosynthetic strategy has been observed for Symbiodiniaceae taxa that
422 are inherently more tolerant of heat and/or light stress (Suggett et al. 2008; Roberty et al. 2014,
423 Pierangelini et al. 2020). This strategy is conducive to a higher overall PSII electron flow
424 (Gorbunov et al. 2011), yet would result in a smaller proportion of electrons that flow linearly
425 to Ci fixation (Cardol et al. 2011). Conversely, reliance on [1 - Q] for reef corals suggests a
426 strategy facilitating dissipation of excess excitation energy upstream of PSII (i.e. rather than
427 relying on O_2-consuming pathways), which could explain the comparatively higher P_G $cell^{-1}$
428 values than mangrove corals. Importantly, light-dependent O_2 consumption was not explicitly
429 measured here, so the extent to which patterns in photobiology observed here can be attributed
430 to this process could warrant further targeted examination. Despite similar light intensity at
431 both sites, micro-light environments and light availability to the symbionts at the cellular level
432 could differ between the two sites. Indeed, mangrove corals harboured 48% more symbionts
433 than those from the reef, potentially inducing self-shading and thus reducing light availability
434 (Schrameyer et al. 2014).
435
436 *P. lutea* exhibited further differences in its metabolic strategy compared to *A. millepora*
437 and *P. acuta* when normalised to symbiont cell density, with a decrease of P_G and an increase
438 of respiration from the reef to the mangrove, but with the same energy-quenching pattern as *A.*
439 *millepora*. However, in this case, symbionts of *P. lutea* from reef and mangrove were

440 conserved from the genus *Cladocopium* but with different ITS2 major types (C15) between
441 habitats. Recent observations reported a similar outcome for major ITS2 type for corals in New
442 Caledonia mangroves and adjacent reefs, whereby *Acropora muricata* always associated with
443 Symbiodiniaceae of the genus *Cladocopium*, but *Acropora pulchra* associated with
444 *Symbiodinium* spp. in the mangrove versus *Cladocopium* spp. on the reef (Camp et al. 2020).
445 Thus, together, these observations suggest numerous "solutions" which corals may use to thrive
446 in mangrove lagoons, via shifts in metabolic strategy that may or may not accompany
447 associations with different Symbiodiniaceae taxa.
448
449 Carbon fixation and translocation in mangrove corals
450 Symbiodiniaceae *in hospite* were less efficient at fixing Ci and translocating
451 photosynthates in the mangrove compared to reef populations. In both habitats, Ci uptake was
452 greatly enhanced by the presence of the host compared to isolated symbionts (see
453 Supplementary Information). Overall, mangrove corals fixed less Ci (29.88% decrease) as
454 demonstrated by stable isotope tracking both at the bulk scale and at the single-cell levels.
455 Mangrove corals also had similar reductions in P_G cell^{-1} compared to the reef (37.80%
456 decrease), suggesting that the loss of Ci in mangrove corals could come from a loss of P_G.
457 However, as the mangrove lagoon is more acidic (and has higher *p*CO2 availability), we
458 expected to observe an increase in productivity (Brading et al. 2011; Suggett et al. 2012b;
459 Hoadley et al. 2015), but in fact measured the opposite. This decrease in Ci uptake therefore
460 might be driven by an upstream pathway independent of CO_2 availability. As physiological
461 processes did not allow for an increased uptake of Ci despite the additional carbon available in
462 the mangrove waters, it is likely that the observed reduction is characteristic of a trade-off
463 linked to stress tolerance. Interestingly, despite a different absolute Ci uptake between corals
464 at the two sites, the proportion of translocated carbon to the host remained the same (~ 22%).
465 These findings shared some consistencies with those of Hoadley et al. (2015), who found that
466 in *Exaiptasia pallida*, increased pCO_2 did not have an effect on carbon translocation, but
467 enhanced Ci fixation by *Breviolum minutum* symbionts. Interestingly, translocation rates of
468 *Cladocopium* sp. in the zoanthid *Palythoa* sp., found by Graham and Sanders (2016) were
469 similar (~26%) to ours (~22%) when the holobionts were incubated at 27°C at a controlled pH
470 of 8.1, which are similar conditions of our incubations (28.0°C at pH 8.02). Moreover, Graham
471 and Sanders (2016) also found that the combination of high temperature (31°C) and high pCO_2
472 (pH 7.65; which are characteristic of mangrove environments) increased productivity and
473 translocation rates (up to 40%) of *Cladocopium* sp. symbionts. In our study, the incubation

474 conditions of temperature and pH were similar between reef and mangrove corals, thus it is
475 possible that the trends observed will be shifted towards a better performance of mangrove
476 holobionts in response to elevated temperature and/or pCO_2 (Mora et al. 2013). Whilst
477 responses to increased pCO_2 levels point toward an enhanced productivity and translocation of
478 Symbiodiniaceae in symbiosis with anemones (Suggett et al. 2012b; Hoadley et al. 2015) and
479 zoanthids (Graham and Sanders 2016), our results suggest that carbon assimilation and
480 transfers are likely to be species-specific, particularly when considering calcifying hosts.

481 Even with considerable long-term environmental differences between mangrove and
482 reef habitats, the proportions of translocated carbon remained similar, possibly due to the host
483 promoting microenvironmental stability surrounding the symbionts. Effect of the host on Ci
484 uptake by the symbiont has already been characterised in previous laboratory-based studies,
485 notably by changing the physicochemical microenvironment surrounding the symbiotic cells
486 inside of the symbiosome (Barott et al. 2015), a specialised vacuole of the coral surrounding
487 the endosymbionts (Roth et al. 1988), however, this has not been directly measured in
488 mangrove corals. Since in the present study both symbiont identity and environments are
489 different, we cannot conclude if *in hospite*, the difference in Ci uptake is solely due to the
490 growth environment or the taxonomic identity. Previous work by Camp et al. (2019) at Low
491 Isles did not measure Ci uptake (or translocation), but their parallel measurements of
492 respirometry and photochemical quenching patterns highlight that each of the three coral
493 species commonly found across the Low Isles reef and mangrove lagoon (*A. millepora*,
494 *P. lutea*, *P. acuta*) exhibit very different metabolic strategies, suggesting that growth
495 environments and both host and symbiont identity could play a role in diversity of Ci uptake
496 and translocation. Quantifying the Ci uptake of *in hospite* symbionts from similar major ITS2
497 types but from different environments (see *P. lutea*; Camp et al. 2019 and *A. muricata* in New
498 Caledonia; Camp et al. 2020) could help in determining the effect of different growth
499 environments on Ci assimilation strategies inherently characteristic of extreme environments.
500 The combination of increased respiratory rates in mangrove compared to the reef, corroborated
501 by a decrease in enriched storage lipid bodies suggests that heterotrophy is likely an additional
502 way of supplementing the energetic requirements of corals in inhospitable conditions (which
503 require an increased energetic demand to sustain homeostasis and survival) (Palardy et al.
504 2008). Such diversity in response presumably reflects diverse resource requirements of
505 different host-coral species (Suggett et al. 2017), how they are able to meet their requirements
506 via heterotrophic supplementation (Fox et al. 2018), and potential co-evolution of host-
507 Symbiodiniaceae associations (Qin et al. 2019; Wright et al. 2019).

508

509 Rapid degradation of coral reefs worldwide has created an urgent need to identify local
510 refuge environments as well as stress-tolerant coral populations that could aid the long-term
511 survival of corals. Our work builds on the growing body of evidence (e.g. Camp et al. 2018;
512 Morikawa and Palumbi 2019; Burt et al. 2020) that corals from extreme environments,
513 including mangrove lagoons, have the potential to act as important stocks of stress-hardened
514 corals. However, our findings highlight costs associated with survival into extreme
515 environments, specifically lower quantities of Ci uptake by Symbiodiniaceae and organic
516 carbon translocated to their coral host, *P. acuta*. Our results confirm that corals exhibit species-
517 specific differences in their adaptation to extremes, highlighting the complexities of resolving
518 stress tolerance to multiple abiotic parameters, characteristic of future global climate change
519 conditions.

520

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532

533 **Author contributions**

534 MR, DJS, EFC and JE designed and conducted all fieldwork and MR conducted experiments;
535 MR and DJH designed the dual fractions incubation chambers, EFC performed respirometry
536 measurements and symbiont identity analysis; DJS collected and analysed the photo-
537 physiological data; MR, DJS, EFC collected the coral samples; MR, MP, EFC and JBR
538 designed the stable isotope labelling experiments and MR performed incubations, cell density
539 measurements, prepared samples for stable isotope analysis and analysed NanoSIMS images
540 with the help of JBR; TH performed host and symbiont DNA extraction for identity analysis;
541 MK, JBR and MP prepared the samples for NanoSIMS analysis; PG and JB performed the

542 NanoSIMS image acquisition. MR, DJS, JBR and EFC wrote the manuscript; all authors
543 provided subsequent editorials.

544 **Conflict of interest**

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

546

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759

760 **Figures**

761

762 **Figure 1.** Physiology of *Pocillopora acuta* (n = 5; mean ± SE) in the Low Isles reef site (blue)
763 and Woody Isles mangrove lagoon (red). (**a**) respiration and gross photosynthesis (P_G) (as the
764 sum of net photosynthesis and respiration) normalised per surface area, and (**b**) per
765 endosymbiont cell, (**c**) photochemical quenching [1 – C] versus non-photochemical quenching
766 [1 – Q]. The asterisk symbol denotes statistical differences ($P < 0.05$). Note: when error bars
767 are not shown, their size are smaller than the symbol used.

768

769 **Figure 2.** Carbon enrichments at the bulk and single-cell levels. (**a**) Bulk (n = 4) $\delta^{13}C$
770 enrichment levels (normalised to natural abundances) for the different incubated fractions of
771 the coral: "Coral" and "Symbiodiniaceae" refer to the incubated holobiont, and later separated
772 algae fractions, respectively. (**b**) Single cell $\delta^{13}C$ enrichment levels with NanoSIMS. The errors
773 bars represent standard error, the asterisk symbols denote statistical differences ($P < 0.05$)
774 between reef (**c-d**) and mangrove (**e-f**) sites. Representative NanoSIMS images showing (**c-e**)
775 the distribution of $^{12}C^{14}N^{-}$, indicative of the biological structure of the sample, and (**d-f**) the
776 isotope ratio of $^{13}C/^{12}C$, with natural abundance in blue, changing to pink with increasing ^{13}C
777 levels. Number of cells analysed: *in hospite* Symbiodiniaceae (n = 19); and enriched areas in
778 host tissue (n = 5). Scale bar: 5 µm.

779

780 **Figure 3.** Relative abundances (%) of recovered ITS2 sequences (upper section) and predicted
781 major ITS type profiles (lower section) for *Pocillopora acuta* across the Low Isles reef and
782 Woody Isles mangrove habitats on the Great Barrier Reef. Each stacked bar corresponds to a
783 biological replicate of a different colony, and each replicate is plotted relative to each other
784 between the upper and lower sections of the figure. Sequences with designated names (e.g.
785 C1b, D4c, or D6) refer to sequences frequently found in the literature or already characterising
786 ITS2 profiles previously ran through the SymPortal analytical framework (Hume et al. 2019).
787 Other sequences designated by a unique database ID and their associated genus (e.g. 70776_D)
788 refer to sequences that are less common and not previously used to characterise ITS type
789 profiles.

790

791 **Figure 4.** Comparisons of (a) mean (n = 4 ± SE) gross photosynthesis (P_G, as the sum of net
792 photosynthesis and respiration, R) and respiration rates between reef (blue dots) and mangrove
793 (red squares) sites. Species-specific metabolic shifts are represented by solid lines (black), and

794 the dashed line indicates the 1:1 ratio. The table summarises the mean (± standard error) P_G:R
795 ratios for reef and mangrove sites. (b) Summary of key metrics shifts from reef and mangrove
796 sites. Data for *Acropora millepora* and *Porites lutea* are retrieved from Camp et al. (2019) and
797 for *Pocillopora acuta* come from the present study. The asterisk symbols denote statistically
798 different shifts ($P < 0.05$), the absence of asterisk symbol denotes trends, the equal signs denote
799 no statistical differences ($P > 0.05$), and NA denotes data not collected.