Baseline dynamics of Symbiodiniaceae genera and photochemical efficiency in corals from reefs with different thermal histories

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Ocean warming and marine heatwaves induced by climate change are impacting coral reefs globally, leading to coral bleaching and mortality. Yet, coral resistance and resilience to warming are not uniform across reef sites and corals can show inter- and intraspecific variability. To understand changes in coral health and to elucidate mechanisms of coral thermal tolerance, baseline data on the dynamics of coral holobiont performance under non-stressed conditions are needed. We monitored the seasonal dynamics of algal symbionts (family Symbiodiniaceae) hosted by corals from a chronically warmed and thermally variable reef compared to a thermally stable reef in southern Taiwan over 15 months. We assessed the genera and photochemical efficiency of Symbiodiniaceae in three coral species: *Acropora nana*, *Pocillopora acuta,* and *Porites lutea*. Both *Durusdinium* and *Cladocopium* were present in all coral species at both reef sites across all seasons, but general trends in their detection (based on qPCR cycle) varied between sites and among species. Photochemical efficiency (i.e., maximum quantum yield; F_v/F_m) was relatively similar between reef sites but differed consistently among species; no clear evidence of seasonal trends in F_v/F_m were found. Quantifying natural Symbiodiniaceae dynamics can help facilitate a more comprehensive interpretation of thermal tolerance response as well as plasticity potential of the coral holobiont.

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42 **Abstract**

43

44 Ocean warming and marine heatwaves induced by climate change are impacting coral reefs

45 globally, leading to coral bleaching and mortality. Yet, coral resistance and resilience to warming

46 are not uniform across reef sites and corals can show inter- and intraspecific variability. To

47 understand changes in coral health and to elucidate mechanisms of coral thermal tolerance,

48 baseline data on the dynamics of coral holobiont performance under non-stressed conditions are

49 needed. We monitored the seasonal dynamics of algal symbionts (family Symbiodiniaceae)

50 hosted by corals from a chronically warmed and thermally variable reef compared to a thermally

51 stable reef in southern Taiwan over 15 months. We assessed the genera and photochemical

52 efficiency of Symbiodiniaceae in three coral species: *Acropora nana*, *Pocillopora acuta*, and

53 *Porites lutea*. Both *Durusdinium* and *Cladocopium* were present in all coral species at both reef

54 sites across all seasons, but general trends in their detection (based on qPCR cycle) varied

55 between sites and among species. Photochemical efficiency (i.e., maximum quantum yield;

56 F_v/F_m) was relatively similar between reef sites but differed consistently among species; no clear

57 evidence of seasonal trends in F_v/F_m were found. Quantifying natural Symbiodiniaceae dynamics

58 can help facilitate a more comprehensive interpretation of thermal tolerance response as well as

59 plasticity potential of the coral holobiont.

60

61

62 **Introduction**

63

64 The health and persistence of corals in the Anthropocene are most highly threatened by climate

65 change (Hoegh-Guldberg et al., 2017; Hughes et al., 2017), in particular ocean warming and

66 marine heatwaves (Spalding & Brown, 2015; Leggat et al., 2019). Elevated seawater

67 temperatures can lead to oxidative stress in corals (Oakley & Davy, 2018), which prompts the

68 expulsion of the coral's symbiotic dinoflagellate algae (family Symbiodiniaceae), from which

69 scleractinian (i.e., reef-building) corals typically obtain most of their energy (Yellowlees, Rees &

70 Leggat, 2008; van Oppen & Lough, 2018). This physiological response leads to coral bleaching

71 and may result in mortality, which can have detrimental effects on reef ecosystems and coral-

72 dependent communities (e.g., phase shifts to algal-dominated states [Ostrander et al., 2000;

73 Vaughan et al., 2021]; homogenization of fish populations [Richardson, Graham & Hoey,

74 2020]).

75

76 The effect of elevated temperature on corals, however, is not uniform among species and sites.

77 Corals with 'weedy' life-history traits (e.g., *Pocillopora* spp.), which typically have a brooding

78 reproductive strategy and are recruitment pioneers, appear to fare better under elevated

79 temperatures than 'competitive' corals (e.g., *Acropora* spp.), which are fast-growing broadcast

80 spawners, but neither performs as well as 'stress-tolerant' corals (e.g., massive *Porites* spp.),

81 which are slow-growing broadcast spawners (Darling, McClanahan & Côté, 2013; Kubicek et

82 al., 2019). In addition, reef site characteristics can also affect coral thermal tolerance (Camp et
83 al., 2018; Burt et al., 2020). Reef sites that have shown higher resistance to bleaching include
84 some that experience chronic disturbance (e.g., Guest et al., 2016), a wide seasonal range of
85 temperatures – including chronic high maximum temperatures (e.g., Riegl & Purkis, 2012)
86 and/or highly variable thermal regimes (e.g., Wyatt et al., 2020). The primary mechanisms that
87 allow some corals to persist and prosper are likely underpinned on a fundamental level by the
88 capacity for effective and consistent energy acquisition. Here we focus on coral performance in
89 relation to the genera and photochemical efficiency of the coral's symbiotic algae.
90
91 The symbiotic relationship between scleractinian corals and Symbiodiniaceae has allowed coral
92 reefs to flourish in nutrient-poor marine environments due to the efficient transfer of
93 photosynthetic products from the symbiont to the coral host (Muscatine, 1990; Roth, 2014).
94 Corals can associate with a range of Symbiodiniaceae genera, often more than one at a time
95 (Baker, 2003; Silverstein, Correa & Baker, 2012), and the genus of Symbiodiniaceae can
96 influence the host's bleaching resistance (Berkelmans & van Oppen, 2006). For example, the
97 genus *Durusdinium* (formerly known as clade D) has been associated with high bleaching
98 resistance under both heat and cold stress (Stat & Gates, 2011; Silverstein, Cunning & Baker,
99 2017), but typically at the cost of slower coral growth compared to the more thermally sensitive
100 genus *Cladocopium* (formerly known as clade C) (Jones & Berkelmans, 2010; LaJeunesse et al.,
101 2018). Symbiodiniaceae genus composition can vary spatially, with corals in more thermally
102 extreme sites generally being more likely to host relatively more *Durusdinium* than other genera
103 (Oliver & Palumbi, 2009; Keshavmurthy et al., 2012; but see Howells et al., 2020 for an example
104 of thermally tolerant *Cladocopium*). Further, species within a genus of Symbiodiniaceae can also
105 show different responses to high temperature, which can vary based on coral host-algal symbiont
106 pairing (Hoadley et al., 2019).
107
108 In general, a challenge to pinpointing potential mechanisms of thermal tolerance in corals is the
109 lack of long-term, *in situ* physiological/molecular data under unstressed conditions (e.g.,
110 relatively poor understanding of seasonal trends and natural fluctuations). Such data are needed
111 to put monitoring studies (e.g., pre- and post-bleaching surveys) and short-term laboratory
112 experiments in context. This is especially pertinent when assessing the potential role of coral
113 traits and reef site characteristics (e.g., temperature regime) on the acquisition of thermal
114 resistance and resilience.
115
116 We assessed the seasonal dynamics of algal symbionts hosted by corals from a chronically
117 warmed and thermally variable reef compared to a thermally stable reef in southern Taiwan over
118 15 months. We examined the dynamics of Symbiodiniaceae genus associations and
119 photochemical efficiency across three common coral species with different life-history traits:
120 *Acropora nana, Pocillopora acuta*, and *Porites lutea*, at both sites. We predicted that in the
121 absence of large thermal anomalies, Symbiodiniaceae genus/genera associations would remain

122 stable across seasons but would show site- and species-specific differences due to the distinct
123 thermal regimes of the reef sites and species life-histories, respectively. We predicted that
124 photochemical efficiency would vary across seasons, but that seasonal trends among species
125 would be similar.
126
127

Materials & Methods
129
130 This study was initially reported in McRae (2021) in partial fulfillment of PhD requirements.
131

Study sites
133 The two study sites, Outlet reef (21.931°E, 120.745°N) and Wanlitung reef (21.955°E,
134 120.766°N), are located in southern Taiwan (**Fig. S1**). Outlet reef is situated within Nanwan Bay
135 and has a highly variable thermal regime (daily temperature variation ~1.5-3.2°C [Carballo-
136 Bolaños et al., 2019]) due to internal tide-induced upwelling (Jan et al., 2004; Keshavmurthy et
137 al., 2019). This reef has been chronically warmed for over three decades due to the warm water
138 discharge from an adjacent nuclear power plant, and therefore experiences higher summer
139 temperatures than other reefs in southern Taiwan (Hung, Huang & Shao, 1998; Keshavmurthy et
140 al., 2014), which has led to acclimatization/adaptation in some corals and their algal symbionts
141 (e.g., Mayfield, Fan & Chen, 2013; Carballo-Bolaños et al., 2019). Wanlitung reef is situated on
142 the western coast of the Hengchun peninsula (~8 km northwest of Outlet reef) and has a
143 relatively stable thermal regime throughout the year (daily temperature variation ~0.7-1.6°C
144 [Carballo-Bolaños et al., 2019]). Both Outlet and Wanlitung reefs have been, and continue to be,
145 impacted by anthropogenic stressors (e.g., tourism and nutrient pollution [Meng et al., 2008; Liu
146 et al., 2012]). Live coral cover in 2010 was ~55% at Outlet reef (Keshavmurthy et al., 2014), and
147 ~17% at Wanlitung reef (Kuo et al., 2012).
148

Monitoring abiotic conditions: temperature and nutrients
150 A temperature logger (HOBO pendant UA-002-08, Onset Computer Corporation, Massachusetts,
151 USA) was deployed at ~3 m depth at each site and recorded seawater temperature every 10 min
152 from June 2018 to August 2019. Some temperature data collected in this study have been
153 published as supplementary information for a complementary laboratory experiment using the
154 same sites and coral species (see McRae et al., 2022). Water chemistry was monitored monthly
155 at each site over approximately the same period following the protocols outlined in Meng et al.
156 (2008). Parameters measured included 5-day biological oxygen demand (BOD_5), nitrite (NO_2^-),
157 nitrate (NO_3^-), ammonia (NH_3), and phosphate (PO_4^{3-}). Water samples were taken monthly from
158 the same location and depth (~3 m) at each site; the two study sites were sampled on the same
159 day and within 1 h of each other.
160

Field collection

162 Ten coral colonies of each of three coral species *A. nana*, *P. acuta*, and *P. lutea*, situated at ~2-4
163 m depth, were tagged at each reef in May 2018. The same colonies were sampled five times
164 (dates refer to Outlet and Wanlitung reefs, respectively): summer 2018 (July 30 and August 1),
165 fall 2018 (October 25; both sites), winter 2019 (February 22 and 21), spring 2019 (April 27 and
166 23), and summer 2019 (August 29 and 27) (Kenting National Park collection permit numbers:
167 1080000091, 1090006457). In the rare instances where a colony had died or could not be
168 relocated, a new colony was tagged and sampled (see **Tables S1 and S2**). Sampling at each site
169 was conducted between ~10:00 to 12:00. Three small pieces (~2 cm in length) from each colony
170 were removed at each time point using shears (*A. nana, P. acuta*) or a hammer and chisel (*P.*
171 *lutea*). Colonies of each species were situated at least 5 m away from each other (minor
172 exceptions to this rule are noted in **Tables S1 and S2**) and intraspecific colony size was similar
173 across sites. Despite repeated sampling on the same colonies, there was no evidence of long-term
174 sampling damage to colonies; indeed, tissue had regrown over the wound sites between time
175 points and there was no visual evidence of disease (CJM, personal observations).
176
177 **DNA extractions and identification of Symbiodiniaceae genera**
178 A subset of six colonies per species from each site was selected for Symbiodiniaceae analysis
179 (see **Tables S3 and S4**) across each of the five time points assessed in this study. Colonies in the
180 subset were selected if they were: 1) situated at least 5 m away from an adjacent colony of the
181 same species, and 2) were collected at each seasonal time point across the study (minor
182 exceptions noted in **Table S4**). While we acknowledge that exclusion of colonies that were not
183 collected at each time point may present a subtle survival bias, we chose this approach because
184 our assessment of seasonal trends necessitated data from each time point. Symbiodiniaceae
185 analyses were successfully conducted on this subset for each season, with the exception of *P.*
186 *lutea* at Outlet (only n = 5 colonies/season) and *P. acuta* (Outlet reef: only n = 5 colonies/season;
187 Wanlitung reef: only n = 2 colonies/season). DNA extraction was accomplished using a salting-
188 out method modified from Ferrara et al. (2006) based on the protocol described in Keshavmurthy
189 et al. (2020). In brief, coral tissue was lysed overnight in a 2 mL Eppendorf tube with 200 μL of
190 lysis buffer (0.25 M Tris, 0.05 M EDTA at pH 8.0, 2% sodium dodecyl sulfate and 0.1 M NaCl)
191 and 10 μL of 10 mg/mL proteinase E at 55°C in a water bath. Then, NaCl (210 μL at 7 M) was
192 added to the lysed tissue, and the sample was mixed by inverting the tube. The solution was
193 subsequently transferred to a 2 mL collection tube with a DNA spin column (Viogene, USA) and
194 centrifuged at 8000 rpm for 1 min. The lysate was washed twice with 500 μL of ethanol (70%)
195 by centrifuging at 8000 rpm for 1 min during each step, with an additional centrifugation step at
196 8000 rpm for 3 min to dry the spin column. The column was dried at 37°C for 15 min, then the
197 DNA was eluted with 50 μL of preheated (65°C) 1X TE buffer, and was centrifuged at 15000 g
198 for 3 min. The quality of genomic DNA was assessed using a 1% agarose gel. The
199 concentrations of genomic DNA were determined using NanoDrop 2000 (Thermal Scientific,
200 USA).
201

202 The presence or absence of *Cladocopium* sp. and *Durusdinium* sp. in coral samples was
203 determined using the primer pairs for *Cladocopium* and *Durusdinium*-specific ITS1 in qPCR
204 assays (Ulstrup & Van Oppen, 2003). The detection cut-off cycle was set to 35 to avoid false
205 positives caused by the formation of non-specific fluorescence (see Mieog et al., 2007). Each
206 qPCR reaction contained 2X Fast Start Universal SYBR Green Master (ROX), 100 nM
207 Symbiodiniaceae universal forward primer and *Cladocopium* or *Durusdinium*-specific reverse
208 primer, 2.0 µL DNA template (similar concentration within species and within seasons), and
209 deionized sterile water to a total volume of 10 µL. Based on the protocol described in
210 Keshavmurthy et al. (2022), amplifications were carried out on an StepOne Plus real-time PCR
211 instrument (Applied Biosystems) with thermal cycling conditions consisting of a denaturation
212 step at 95°C of 10 min followed by 35 two-step cycles of 15 s at 95°C and 1 min at 60°C. At the
213 end of each run, a melt curve generated by temperature elevation from 60°C to 95°C in 0.5°C
214 increments each 5 s for 70 cycles was included to ensure that only target sequences were
215 amplified. All qPCR reactions were run in triplicate (technical replicates), as was a no-template
216 control (NTC) with ddH2O. It is important to note that specific quantification of the composition
217 of each Symbiodiniaceae genus (i.e., dominant vs. background genus) was not conducted in this
218 study. Instead, we provide a coarse assessment of the presence/absence of Symbiodiniaceae upon
219 which we hope future research can investigate in more detail.
220
221 **Maximum quantum yield (F_v/F_m) and sample fixation**
222 Coral fragments were immediately transported to the research facilities of the National Museum
223 of Marine Biology and Aquarium (Pingtung County, Taiwan) for sampling and photochemistry
224 measurements. Corals were held in coolers filled with seawater from the source reef during
225 transportation; transit duration was approximately 10 min (Wanlitung reef) or 20 min (Outlet
226 reef). One fragment from each colony was dark adapted for 30 min and three replicate
227 measurements of maximum quantum yield (F_v/F_m), a measure of photochemical efficiency
228 (Jones et al., 2000), were taken using a diving PAM (Heinz Walz GmbH, Germany; settings:
229 saturation pulse intensity = 8, measurement light intensity= 8, gain = 2, damp = 2).
230 Measurements of F_v/F_m were undertaken at our lab facilities (rather than in the field) to allow for
231 effective dark adaptation, and because accurate *in situ* measurements would have been
232 challenging during summer sampling due to the high wave action from typhoons and tropical
233 storms commonly affecting the waters surrounding Taiwan. Another fragment from each colony
234 was placed in ethanol for Symbiodiniaceae genera assessment. The last fragment from each
235 colony was placed in liquid nitrogen for lipid assessment, but due to an error in sample storage
236 (i.e., stored at -20°C, instead of -80°C) we have omitted these data due to concerns of possible
237 sample degradation. We do, however, think it is important to mention our error here as a
238 cautionary tale; see Vega Thurber et al. (2022) for a recent and comprehensive review of optimal
239 sample fixation and storage protocols.
240
241 **Statistical analysis**

242   We used linear mixed-effects models (and post-hoc pairwise comparisons) to assess (1) the
243   effect of reef site and season, and their interaction, on water temperature (with month as a
244   random effect to mitigate temporal autocorrelation), and (2) the effect of reef site, season, and
245   coral species, and their interactions, on F_v/F_m (with colony as a random effect to account for
246   repeated sampling). Linear mixed-effects model assumptions were visually checked by plotting
247   model residuals and through calculation of generalized variance inflation factors;
248   GVIF^(1/(2*df) was used to calculate generalized variation inflation factors as this approach is
249   more suitable for categorical variables. We report the presence/absence of *Cladocopium* and
250   *Durusdinium* detected in coral colonies at each site across seasons for each species. We used
251   either a Student's t-test or a Mann-Whitney U test (depending on data distribution) to assess
252   difference between reef sites for each water chemistry parameter; model assumptions of
253   normality and equal variance were tested using Shapiro-Wilk and Levene's tests, respectively.
254   All analyses were conducted in R (R Core Team, 2022) using the packages: lme4 (Bates et al.,
255   2015), lmerTest (Kuznetsova, Brockhoff & Christensen, 2017), emmeans (Length, 2022), car
256   (Fox & Weisberg, 2019), and lubridate (Grolemund & Wickham). All data and R scripts used in
257   our analyses are publicly available on GitHub at: https://github.com/CJ-McRae/McRae-et-
258   al_Peer-J-submission, and on Zenodo at https://doi.org/10.5281/zenodo.7762107.

259

Results

261

Reef site temperature and nutrients

263   The mean temperature (± SD) at Outlet and Wanlitung reefs over the study period of June 2018-
264   August 2019 was 27.9 ± 1.8˚C and 27.8 ± 1.7˚C, respectively (**Fig.1A & B**). Mean daily
265   temperature was higher at Outlet than Wanlitung reef in winter (linear mixed-effects model,
266   post-hoc pairwise comparison; $t = 3.59$, $p = 0.01$) and spring ($t = 7.61$, $p < 0.001$) (**Fig. 1C**).
267   Outlet reef had consistently larger daily temperature ranges (2.85 ± 1.25˚C) than Wanlitung (1.27
268   ± 0.59˚C) across each season (all $t \geq 5.12$, all $p < 0.001$) (**Fig. 1D**). Outlet reef had higher
269   maximum temperatures across all seasons (all $t \geq 5.69$, all $p < 0.001$), with the exception of fall
270   2018 when no difference was found between sites (**Fig. 1E**). Lower temperature minima were
271   also found at Outlet reef in summer 2018 ($t = -11.86$, $p < 0.001$) and summer 2019 ($t = -8.22$, all
272   $p < 0.001$) (**Fig. 1F**). Water chemistry parameters did not differ between reef sites (Mann
273   Whitney U tests, all $W \leq 79$, all $p > 0.05$ for BOD_5, NO_2^-, NH_3, and PO_4^{3-}; t-test, $t = 0.46$, $p >$
274   0.05 for NO_3^-) (**Table S5**). In brief, the means (± SD) for Outlet and Wanlitung reefs,
275   respectively, were 1.0 ± 0.4 and 1.1 ± 0.6 for BOD_5, 0.006 ± 0.015 and 0.003 ± 0.005 for NO_2^-,
276   0.017 ± 0.007 and 0.018 ± 0.007 for NH_3, 0.006 ± 0.005 and 0.011 ± 0.028 for PO_4^{3-}, and 0.017
277   ± 0.014 and 0.014 ± 0.010 for NO_3.

278

Symbiodiniaceae associations

280   All corals hosted a combination of both Symbiodiniaceae genera across all seasons at both sites
281   (**Fig. 2-4**). In general, the detection of the genus of Symbiodiniaceae (i.e., based on qPCR

282 amplification cycle) showed site- and species-specific patterns that remained relatively consistent
283 across seasons. Colonies of *A. nana* at Outlet reef (i.e., the warmed and thermally variable reef)
284 showed the presence of both *Cladocopium* and *Durusdinium* starting at ~15 qPCR cycles,
285 whereas *A. nana* from Wanlitung reef (i.e., the thermally stable reef) showed the presence of
286 *Cladocopium* at ~15 qPCR cycles and *Durusdinium* at $\geq$ 30 qPCR cycles (**Fig. 2**). In *P. acuta*,
287 *Durusdinium* was detected earlier (~15 qPCR cycles) than *Cladocopium* ($\geq$ 25 qPCR cycles) in
288 colonies from Outlet reef, but the opposite pattern was observed for colonies from Wanlitung
289 reef (i.e., *Cladocopium* detected at ~15 qPCR cycles and *Durusdinium* detected at $\geq$ 25 qPCR
290 cycles) (**Fig. 3**). In *P. lutea*, *Cladocopium* was detected at ~15 qPCR cycles in colonies from
291 both reef sites, but *Durusdinium* was detected earlier ($\geq$ 20 qPCR cycles) in colonies from Outlet
292 reef than Wanlitung reef ($\geq$ 30 qPCR cycles) (**Fig. 4**). Coral samples for the summer 2019 time
293 point were not analyzed due to subsequent COVID-19 restrictions to laboratory access.
294

295 **Maximum quantum yield (F_v/F_m)**
296 Intraspecific differences in F_v/F_m between reef sites occurred periodically in some seasons, with
297 colonies from Outlet reef (i.e., the warmed and thermally variable reef) having higher F_v/F_m than
298 colonies from Wanlitung reef (i.e., the thermally stable reef) (**Fig. 5A-C**). This was the case for
299 *A. nana*, in fall 2018 and summer 2019 (linear mixed-effects model, post hoc pairwise
300 comparisons; both $t \geq 2.47$, $p \leq 0.01$), for *P. acuta* in fall 2018 and spring 2019 (both $t \geq 3.00$, p
301 ≤ 0.003), and for *P. lutea* in summer 2018 only ($t = 2.06$, $p = 0.04$).
302

303 Interspecific differences in F_v/F_m showed similar trends at both reef sites. Colonies of *P. acuta*
304 and *A. nana* did not differ in F_v/F_m in any season, and both species periodically had higher F_v/F_m
305 than *P. lutea* (mean $\pm$ SD across all seasons; Outlet reef, *A. nana*: 0.73 $\pm$ 0.03, *P. acuta*: 0.75 $\pm$
306 0.03, *P. lutea*: 0.63 $\pm$ 0.04; Wanlitung reef, *A. nana*: 0.71 $\pm$ 0.04, *P. acuta*: 0.71 $\pm$ 0.03, *P. lutea*:
307 0.63 $\pm$ 0.03) (**Fig. 5A-C**). At Outlet reef, both *P. acuta* and *A. nana* had higher F_v/F_m than *P.*
308 *lutea* in fall 2018, winter 2019, spring 2019, and summer 2019 (linear mixed-effects model, post-
309 hoc pairwise comparisons; all $t \geq 2.58$, all $p < 0.03$) but only *P. acuta* was higher than *P. lutea* in
310 summer 2018 ($t = 4.29$, $p = 0.001$). At Wanlitung reef, both *P. acuta* and *A. nana* had higher
311 F_v/F_m than *P. lutea* in summer 2018, winter 2019, spring 2019, and summer 2019 (all $t \geq 3.23$, all
312 $p \leq 0.004$), but only *P. acuta* was higher than *P. lutea* in summer 2018 ($t = 4.00$, $p < 0.001$).
313 There was no difference in F_v/F_m among species at Wanlitung reef in fall 2018.
314

315 Overall, there were no clear seasonal trends in F_v/F_m but some variation over time was observed
316 in each species at each site (**Fig. 5A-C**). In *A. nana*, F_v/F_m was higher in spring 2019 than
317 summer 2018 at Outlet reef (linear mixed-effects model, post-hoc pairwise comparisons; $t =$
318 3.61, $p = 0.003$), whereas at Wanlitung reef F_v/F_m was higher in spring 2019 than all other
319 seasons (all $t \geq 3.67$, all $p \leq 0.003$). In *P. acuta*, F_v/F_m was higher in spring 2019 than summer
320 2019 at Outlet reef ($t = 3.56$, $p = 0.004$), in contrast to Wanlitung reef where F_v/F_m was only
321 higher in winter 2019 compared to fall 2018 ($t = 3.57$, $p = 0.004$). In *P. lutea*, F_v/F_m at Outlet reef

322   was lower in summer 2019 than all other seasons except for spring 2019, and was higher in fall
323   2018 compared to spring 2019 (all $t \geq 2.99$, all $p \leq 0.024$), whereas at Wanlitung reef F_v/F_m was
324   higher in fall 2018 and spring 2019 than in summer 2019 (both $t \geq 3.34$, both $p \leq 0.035$).
325
326

327 **Discussion**

328

329   Our comparison of algal symbiont dynamics in *A. nana*, *P. acuta*, and *P. lutea* colonies at a
330   warmed and thermally variable reef (Outlet reef) and a thermally stable reef (Wanlitung reef)
331   showed site- and species-specific patterns. In general, *Durusdinium* was detected earlier in the
332   qPCR cycle than *Cladocopium* in colonies from the warmed and thermally variable reef than at
333   the thermally stable reef. Symbiodiniaceae associations remained relatively consistent across
334   seasons. Reef site patterns in Symbiodiniaceae associations were not clearly mirrored by similar
335   site patterns in photochemical efficiency. Intraspecific F_v/F_m differed between sites in only one
336   or two seasons for each coral species, with corals from the warmed and thermally variable reef
337   periodically having higher F_v/F_m. Interspecific differences showed similar patterns at both sites,
338   with *A. nana* and *P. acuta* typically having higher F_v/F_m than *P. lutea*. Although there was some
339   subtle variation in F_v/F_m over time for each species at each site, no consistent seasonal trends in
340   F_v/F_m were observed.

341

342   **Temperature and nutrients: reef site comparison**
343   The main physical difference between the two reef sites, based on the parameters assessed in this
344   study, was the thermal regime (**Fig. 1**). Outlet reef had higher maximum temperatures,
345   particularly in summer months, than Wanlitung reef as the former is chronically influenced by
346   warm-water effluent from an adjacent nuclear power plant. However, Outlet reef also had lower
347   daily temperature minima due to cold-water upwelling in Nanwan Bay (Hsu et al., 2020). As
348   upwelling somewhat mitigated the power plant warming, mean daily temperature at Outlet reef
349   was higher than at Wanlitung reef only in winter and spring. Corals that experience high
350   variability or extremes in temperature have been shown to have increased thermal tolerance
351   owing to acclimatization and/or adaptation (e.g., genetic adaptations [Barshis et al., 2013],
352   morphological adaptations [Enríquez et al., 2017], hosting thermally tolerant algal symbionts
353   [Oliver & Palumbi, 2011]; but also see [Le Nohaïc et al., 2017; Smith et al., 2017; Klepac &
354   Barshis, 2020] for limits on adaptation in corals from variable/extreme reefs). In contrast, reefs
355   with relatively stable thermal regimes tend to have corals that are more susceptible to bleaching
356   under elevated temperatures (Thomas et al., 2018; Safaie et al., 2018).

357

358   In contrast to temperature regime patterns, nutrient concentrations at both sites were similar
359   across the study period (**Table S5**). Levels of BOD_5, NO_3^-, NO_2^-, NO_3^-, NH_3, and PO_4^{3-} measured
360   across seasons did not differ between Outlet and Wanlitung reefs and were relatively low in
361   comparison to other reefs impacted by anthropogenic stressors in southern Taiwan (Meng et al.,

362 2008; Liu et al., 2012). Performance patterns of corals between sites are therefore more likely to
363 be attributable to differences in thermal regime than to nutrient levels.
364
365 **Symbiodiniaceae dynamics**
366 We found differing patterns in Symbiodiniaceae genera detection between our thermally distinct
367 reef sites (**Fig. 2-4**). Each of the three coral species showed earlier detection (based on qPCR
368 cycle) of the more thermally tolerant *Durusdinium* algal symbiont at the warmed and thermally
369 variable reef than at the thermally stable reef. The presence/absence (and also the proportional
370 composition) of Symbiodiniaceae genera in coral tissues can change after stress events (e.g.,
371 bleaching) with more thermally tolerant genera typically replacing thermally sensitive ones
372 (Jones et al., 2008; Cunning, Silverstein & Baker, 2015, but see Kao et al., 2018; Rouzé et al.,
373 2019, for examples of limited shuffling). It is probable that the chronic warming influence, and
374 associated high summer maximum temperatures, at Outlet reef have resulted in a shift to corals
375 potentially hosting more thermally tolerant Symbiodiniaceae. Indeed, 16 coral genera (including
376 the three genera considered in our study) have been found to predominantly associate with either
377 *Durusdinium* or a combination of *Cladocopium* and *Durusdinium* at Outlet reef (Keshavmurthy
378 et al., 2014). In contrast, the same genera at nearby reefs not influenced by the nuclear power
379 plant, and corals deeper than 7 m at Outlet reef and hence out of range of the warm effluent,
380 predominantly host *Cladocopium* (Keshavmurthy et al., 2014). Potentially associating with more
381 *Durusdinium* may allow corals at Outlet reef to resist bleaching under high summer temperature
382 maxima and/or facilitate their capacity to recover from bleaching (Silverstein, Cunning & Baker,
383 2015). Corals at Wanlitung reef, like many Indo-Pacific coral species that live under more stable
384 conditions, may tend to preferentially host *Cladocopium* because it is an abundant and species-
385 rich Symbiodiniaceae (LaJeunesse et al., 2018) that is not associated with the energetic trade-offs
386 of hosting the more thermally tolerant *Durusdinium* (e.g., Jones & Berkelmans, 2011). We did
387 not explicitly quantify dominant or background levels of Symbiodiniaceae genera in this study,
388 but these observed patterns in qPCR detection would likely benefit from a deeper examination
389 (e.g., moving beyond genus-level to species-level assessment is increasingly viewed as important
390 for improving our understanding of coral-algal symbiont dynamics; see Davies et al., 2023).
391
392 In general, Symbiodiniaceae genera associations remained relatively stable over time for each
393 species and reef site (see also Epstein et al., 2019). This seasonal stability reflects the fact that
394 temperatures remained relatively moderate (i.e., within typical site-specific seasonal ranges)
395 throughout our study, with no heatwaves or summer mass bleaching observed. Symbiodiniaceae
396 genus fidelity may also be attributed to acclimatization/adaptation to reef site characteristics
397 (e.g., Iglesias-Prieto et al., 2004; Howells et al., 2020), long-standing co-evolution of host and
398 symbionts (Thornhill et al., 2014; Turnham et al., 2021), and/or to a species-specific
399 'Symbiodiniaceae signature' to the host colony (Rouzé et al., 2019).
400
401 **Photochemical efficiency dynamics**

402   We examined algal symbiont photochemical efficiency dynamics by tracking F_v/F_m across coral
403   species and seasons at both reef sites (**Fig. 5**). Values of F_v/F_m usually decrease in response to
404   elevated temperature (Jones et al., 2000; Okamoto et al., 2005; Silverstein, Cunning & Baker,
405   2015) and can vary seasonally under non-stressed conditions (Warner et al., 2002), often as a
406   response to annual fluctuation in solar irradiance (Winters, Loya & Beer, 2006). However, we
407   found few intraspecific differences in F_v/F_m between sites, and inconsistent seasonal trends in
408   F_v/F_m among species. Occasional differences between reef sites and seasons were also found by
409   Carballo-Bolaños et al. (2019) who showed that F_v/F_m in the brain coral *Leptoria phrygia* was
410   lower at Wanlitung than Outlet reef (in summer and winter, but not spring), and seasonally
411   variable at Wanlitung reef but not at Outlet reef. In our study, typical seasonal differences in
412   temperature and light may not have been enough to elicit significant changes in F_v/F_m for our
413   coral species. Future investigation into other photochemical metrics may be useful to better
414   assess photosystem dynamics (e.g., Ragni et al., 2010). Alternatively, clear patterns in F_v/F_m
415   seasonality may have been masked by subtle seasonal fluctuations in Symbiodiniaceae
416   composition, i.e., due to differential photochemical performance across genera (Kemp et al.,
417   2014).
418
419   We did nevertheless observe relatively consistent interspecific differences in F_v/F_m. At our sites,
420   during each season, the F_v/F_m for all coral species was within a typical healthy range for coral
421   species in southern Taiwan (Putnam, Edmunds & Fan, 2010; Mayfield, Fan & Chen, 2013;
422   Carballo-Bolaños et al., 2019), but *A. nana* and *P. acuta* had higher F_v/F_m than *P. lutea* in most
423   seasons. Differences in F_v/F_m among species are not uncommon (e.g., higher F_v/F_m in unstressed
424   *Pocillopora meandrina* compared to *Porites rus* [Putnam & Edmunds]; wider thermal breadth in
425   *Porites cylindrica* than *Acropora valenciennesi* [Jurriaans & Hoogenboom, 2020]). This is likely
426   a result of taxon-specific traits, such as coral tissue thickness (Anthony & Hoegh-Guldberg,
427   2003), algal symbiont position within the coral tissue (Edmunds, Putnam & Gates, 2012), and/or
428   genus-specific symbiont associations (Wang et al., 2012; Yuyama et al., 2016).
429
430
431   **Conclusions**
432   We did not detect clear evidence of seasonal trends in dominant Symbiodiniaceae genera or
433   photochemical efficiency in our study species, rather differences were more apparent among
434   sites with contrasting thermal regimes and among coral species – highlighting the importance of
435   species-specific studies. Reef site patterns that we observed in Symbiodiniaceae genera
436   detection, using a coarse presence/absence qPCR approach, merit more comprehensive
437   investigation (i.e., genera or species-level quantification) to better assess the influence of thermal
438   regime on Symbiodiniaceae associations among coral hosts. Baseline seasonal data under non-
439   stressed conditions are pertinent to improve our understanding of energy provision sources
440   relevant to coral thermal tolerance. Identifying typical ranges of normal variability in coral and
441   Symbiodiniaceae physiology, coupled with an appreciation of the role that species traits and reef

442 characteristics play, will allow for a better understanding of coral holobiont resistance and
443 resilience in a warming ocean.
444
445

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References

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455 Anthony KRN, Hoegh-Guldberg O. 2003. Variation in coral photosynthesis, respiration and
456   growth characteristics in contrasting light microhabitats: an analogue to plants in forest
457   gaps and understoreys?: *Habitat light variation and coral photophysiology*. *Functional
458   Ecology* 17:246–259. DOI: 10.1046/j.1365-2435.2003.00731.x.

459 Baker AC. 2003. Flexibility and specificity in coral-algal symbiosis: diversity, ecology, and
460   biogeography of *Symbiodinium*. *Annual Review of Ecology, Evolution, and Systematics*
461   34:661–689. DOI: 10.1146/annurev.ecolsys.34.011802.132417.

462 Barshis DJ, Ladner JT, Oliver TA, Seneca FO, Traylor-Knowles N, Palumbi SR. 2013. Genomic
463   basis for coral resilience to climate change. *Proceedings of the National Academy of
464   Sciences* 110:1387–1392. DOI: 10.1073/pnas.1210224110.

465 Bates D, Maechler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4.
466   *Journal of Statistical Software* 67:1–48. DOI: 10.18637/jss.v067.i01.

467 Berkelmans R, van Oppen MJH. 2006. The role of zooxanthellae in the thermal tolerance of
468   corals: a 'nugget of hope' for coral reefs in an era of climate change. *Proceedings of the
469   Royal Society B: Biological Sciences* 273:2305–2312. DOI: 10.1098/rspb.2006.3567.

470 Burt JA, Camp EF, Enochs IC, Johansen JL, Morgan KM, Riegl B, Hoey AS. 2020. Insights
471   from extreme coral reefs in a changing world. *Coral Reefs* 39:495–507. DOI:
472   10.1007/s00338-020-01966-y.

473 Camp EF, Schoepf V, Mumby PJ, Hardtke LA, Rodolfo-Metalpa R, Smith DJ, Suggett DJ. 2018.
474   The future of coral reefs subject to rapid climate change: lessons from natural extreme
475   environments. *Frontiers in Marine Science* 5:4. DOI: 10.3389/fmars.2018.00004.

476 Carballo-Bolaños R, Denis V, Huang Y-Y, Keshavmurthy S, Chen CA. 2019. Temporal
477   variation and photochemical efficiency of species in Symbiodinaceae associated with
478   coral *Leptoria phrygia* (Scleractinia; Merulinidae) exposed to contrasting temperature
479   regimes. *PLOS ONE* 14:e0218801. DOI: 10.1371/journal.pone.0218801.

480 Cunning R, Silverstein RN, Baker AC. 2015. Investigating the causes and consequences of
481   symbiont shuffling in a multi-partner reef coral symbiosis under environmental change.

Proceedings of the Royal Society B: Biological Sciences 282:20141725. DOI: 10.1098/rspb.2014.1725.

Darling ES, McClanahan TR, Côté IM. 2013. Life histories predict coral community disassembly under multiple stressors. *Global Change Biology* 19:1930–1940. DOI: 10.1111/gcb.12191.

Davies S, Gamache MH, Howe-Kerr LI, Kriefall NG, Baker AC, Banaszak AT, Bay LK, Bellantuono AJ, Bhattacharya D, Chan CX, Claar DC, Coffroth MA, Cunning R, Davy SK, del Campo J, Diaz-Almeyda EM, Frommlet JC, Fuess LE, Gonzalez-Pech RA, Goulet TL, Hoadley KD, Howells EJ, Hume BCC, Kemp DW, Kenkel CD, Kitchen SA, LaJeunesse TC, Lin S, McIlroy S, McMinds R, Nitschke MR, Oakley CA, Peixoto RS, Prada C, Putnam HM, Quigley K, Reich HG, Reimer JD, Rodriguez-Lanetty M, Rosalas S, Saad OS, Sampayo EM, Santos S, Shoguchi E, Smith EG, Stat M, Stephens TG, Strader ME, Suggett DJ, Swain TD, Tran C, Traylor-Knowles N, Voolstra CR, Warner ME, Weis VM, Wright R, Xiang T, Yamashita H, Ziegler M, Correa AM, Parkinson JE. 2023. Building Consensus around the Assessment and Interpretation of Symbiodiniaceae Diversity. *PeerJ*. DOI: 10.7717/peerj.15023.

Edmunds PJ, Putnam HM, Gates RD. 2012. Photophysiological consequences of vertical stratification of *Symbiodinium* in tissue of the coral *Porites lutea*. *The Biological Bulletin* 223:226–235. DOI: 10.1086/BBLv223n2p226.

Enríquez S, Méndez ER, Hoegh-Guldberg O, Iglesias-Prieto R. 2017. Key functional role of the optical properties of coral skeletons in coral ecology and evolution. *Proceedings of the Royal Society B: Biological Sciences* 284:20161667. DOI: 10.1098/rspb.2016.1667.

Epstein HE, Smith HA, Cantin NE, Mocellin VJL, Torda G, van Oppen MJH. 2019. Temporal Variation in the microbiome of *Acropora* coral species does not reflect seasonality. *Frontiers in Microbiology* 10:1775. DOI: 10.3389/fmicb.2019.01775.

Ferrara G, Murgia B, Parodi A, Valisano L, Cerrano C, Palmisano G, Bavestrello G, Sara M. 2006. The assessment of DNA from marine organisms via a modified salting-out protocol. *Cellular and Molecular Biology Letters* 11. DOI: 10.2478/s11658-006-0013-7.

Fox J, Weisberg S. 2019. *An {R} Companion to Applied Regression*. Thousand Oaks, California: Sage.

Grolemund G, Wickham H. Dates and times made easy with lubridate. *Journal of Statistical Software*: 1–25. DOI: 10.18637/jss.v040.i03.

Guest JR, Tun K, Low J, Vergés A, Marzinelli EM, Campbell AH, Bauman AG, Feary DA, Chou LM, Steinberg PD. 2016. 27 years of benthic and coral community dynamics on turbid, highly urbanised reefs off Singapore. *Scientific Reports* 6:36260. DOI: 10.1038/srep36260.

Hoadley KD, Lewis AM, Wham DC, Pettay DT, Grasso C, Smith R, Kemp DW, LaJeunesse TC, Warner ME . 2019. Host–symbiont combinations dictate the photo-physiological response of reef-building corals to thermal stress. *Scientific Reports* 9: 9985. DOI: 10.1038/s41598-019-46412-4

523   Hoegh-Guldberg O, Poloczanska ES, Skirving W, Dove S. 2017. Coral reef ecosystems under
524   climate change and ocean acidification. *Frontiers in Marine Science* 4:158. DOI:
525   10.3389/fmars.2017.00158.

526   Howells EJ, Bauman AG, Vaughan GO, Hume BCC, Voolstra CR, Burt JA. 2020. Corals in the
527   hottest reefs in the world exhibit symbiont fidelity not flexibility. *Molecular Ecology*
528   29:899–911. DOI: 10.1111/mec.15372.

529   Hsu P-C, Lee H-J, Zheng Q, Lai J-W, Su F-C, Ho C-R. 2020. Tide-induced periodic sea surface
530   temperature drops in the coral reef area of Nanwan Bay, Southern Taiwan. *Journal of*
531   *Geophysical Research: Oceans*: e2019JC015226. DOI:
532   https://doi.org/10.1029/2019JC015226.

533   Hughes TP, Barnes ML, Bellwood DR, Cinner JE, Cumming GS, Jackson JBC, Kleypas J, van
534   de Leemput IA, Lough JM, Morrison TH, Palumbi SR, van Nes EH, Scheffer M. 2017.
535   Coral reefs in the Anthropocene. *Nature* 546:82–90. DOI: 10.1038/nature22901.

536   Hung T-C, Huang C-C, Shao K-T. 1998. Ecological survey of coastal water adjacent to nuclear
537   power plants in Taiwan. *Chemistry and Ecology* 15:129–142. DOI:
538   10.1080/02757549808037625.

539   Iglesias-Prieto R, Beltrán VH, LaJeunesse TC, Reyes-Bonilla H, Thomé PE. 2004. Different
540   algal symbionts explain the vertical distribution of dominant reef corals in the eastern
541   Pacific. *Proceedings of the Royal Society of London. Series B: Biological Sciences*
542   271:1757–1763. DOI: 10.1098/rspb.2004.2757.

543   Jan S, Chen C-TA, Tu Y-Y, Tsai H-S. 2004. Physical properties of thermal plumes from a
544   nuclear power plant in the Southernmost Taiwan. *Journal of Marine Science and*
545   *Technology* 12. DOI: 10.51400/2709-6998.2265.

546   Jones A, Berkelmans R. 2010. Potential costs of acclimatization to a warmer climate: growth of a
547   reef coral with heat tolerant vs. sensitive symbiont types. *PLoS ONE* 5:e10437. DOI:
548   10.1371/journal.pone.0010437.

549   Jones AM, Berkelmans R. 2011. Tradeoffs to thermal acclimation: energetics and reproduction
550   of a reef coral with heat tolerant *Symbiodinium* Type-D. *Journal of Marine Biology*
551   2011:1–12. DOI: 10.1155/2011/185890.

552   Jones AM, Berkelmans R, van Oppen MJH, Mieog JC, Sinclair W. 2008. A community change
553   in the algal endosymbionts of a scleractinian coral following a natural bleaching event:
554   field evidence of acclimatization. *Proceedings of the Royal Society B: Biological*
555   *Sciences* 275:1359–1365. DOI: 10.1098/rspb.2008.0069.

556   Jones RJ, Ward S, Amri AY, Hoegh-Guldberg O. 2000. Changes in quantum efficiency of
557   Photosystem II of symbiotic dinoflagellates of corals after heat stress, and of bleached
558   corals sampled after the 1998 Great Barrier Reef mass bleaching event. *Marine and*
559   *Freshwater Research* 51:63. DOI: 10.1071/MF99100.

560   Jurriaans S, Hoogenboom M. 2020. Seasonal acclimation of thermal performance in two species
561   of reef-building corals. *Marine Ecology Progress Series* 635:55–70. DOI:
562   10.3354/meps13203.

563 Kao K-W, Keshavmurthy S, Tsao C-H, Wang J-T, Chen A. 2018. Repeated and prolonged
564   temperature anomalies negate Symbiodiniaceae genera shuffling in the coral *Platygyra*
565   *verweyi* (Scleractinia; Merulinidae). *Zoological Studies*: 14.

566 Kemp DW, Hernandez-Pech X, Iglesias-Prieto R, Fitt WK, Schmidt GW. 2014. Community
567   dynamics and physiology of *Symbiodinium* spp. before, during, and after a coral
568   bleaching event. *Limnology and Oceanography* 59:788–797. DOI:
569   10.4319/lo.2014.59.3.0788.

570 Keshavmurthy S, Hsu C-M, Kuo C-Y, Meng P-J, Wang J-T, Chen CA. 2012. Symbiont
571   communities and host genetic structure of the brain coral *Platygyra verweyi*, at the outlet
572   of a nuclear power plant and adjacent areas: coral acclimation to long-term stress.
573   *Molecular Ecology* 21:4393–4407. DOI: 10.1111/j.1365-294X.2012.05704.x.

574 Keshavmurthy S, Meng P-J, Wang J-T, Kuo C-Y, Yang S-Y, Hsu C-M, Gan C-H, Dai C-F, Chen
575   CA. 2014. Can resistant coral- *Symbiodinium* associations enable coral communities to
576   survive climate change? A study of a site exposed to long-term hot water input. *PeerJ*
577   2:e327. DOI: 10.7717/peerj.327.

578 Keshavmurthy S, Kuo C-Y, Huang Y-Y, Carballo-Bolaños R, Meng P-J, Wang J-T, Chen CA.
579   2019. Coral reef resilience in Taiwan: lessons from long-term ecological research on the
580   coral reefs of Kenting National Park (Taiwan). *Journal of Marine Science and*
581   *Engineering* 7:388. DOI: 10.3390/jmse7110388.

582 Keshavmurthy S, Tee HS, Kao KW, Wang JT, Chen CA. 2020. Specificity trumps flexibility—
583   location-based stable associations between Symbiodiniaceae genera and *Platygyra*
584   *verweyi* (Scleractinia; Merulinidae). *PeerJ* 8:e8791. DOI: 10.7717/peerj.8791.

585 Keshavmurthy S, Chen TR, Liu PJ, Wang JT, Chen CA. 2022. Learning from the past is not
586   enough to survive present and future bleaching threshold temperatures. *Science of The*
587   *Total Environment* 852: 158379. DOI: 10.1016/j.scitotenv.2022.158379.

588 Klepac CN, Barshis DJ. 2020. Reduced thermal tolerance of massive coral species in a highly
589   variable environment. *Proceedings of the Royal Society B: Biological Sciences*
590   287:20201379. DOI: 10.1098/rspb.2020.1379.

591 Kubicek A, Breckling B, Hoegh-Guldberg O, Reuter H. 2019. Climate change drives trait-shifts
592   in coral reef communities. *Scientific Reports* 9:3721. DOI: 10.1038/s41598-019-38962-4.

593 Kuo C-Y, Yuen YS, Meng P-J, Ho P-H, Wang J-T, Liu P-J, Chang Y-C, Dai C-F, Fan T-Y, Lin
594   H-J, Baird AH, Chen CA. 2012. Recurrent disturbances and the degradation of hard coral
595   communities in Taiwan. *PLoS ONE* 7:e44364. DOI: 10.1371/journal.pone.0044364.

596 Kuznetsova A, Brockhoff P, Christensen R. 2017. lmerTest Package: Tests in linear mixed
597   effects models. *Journal of Statistical Software* 82:1–26. DOI: 10.18637/jss.v082.i13.

598 LaJeunesse TC, Parkinson JE, Gabrielson PW, Jeong HJ, Reimer JD, Voolstra CR, Santos SR.
599   2018. Systematic revision of Symbiodiniaceae highlights the antiquity and diversity of
600   coral endosymbionts. *Current Biology* 28:2570-2580.e6. DOI:
601   10.1016/j.cub.2018.07.008.

602 Le Nohaïc M, Ross CL, Cornwall CE, Comeau S, Lowe R, McCulloch MT, Schoepf V. 2017.
603   Marine heatwave causes unprecedented regional mass bleaching of thermally resistant

604 corals in northwestern Australia. *Scientific Reports* 7:14999. DOI: 10.1038/s41598-017-
605 14794-y.

606 Leggat WP, Camp EF, Suggett DJ, Heron SF, Fordyce AJ, Gardner S, Deakin L, Turner M,
607 Beeching LJ, Kuzhiumparambil U, Eakin CM, Ainsworth TD. 2019. Rapid coral decay is
608 associated with marine heatwave mortality events on reefs. *Current Biology* 29:2723-
609 2730.e4. DOI: 10.1016/j.cub.2019.06.077.

610 Length R. 2022. emmeans: Estimated Marginal Means, aka Least-Squares Means. R package
611 version 1.7.4-1.

612 Liu P-J, Meng P-J, Liu L-L, Wang J-T, Leu M-Y. 2012. Impacts of human activities on coral
613 reef ecosystems of southern Taiwan: A long-term study. *Marine Pollution Bulletin*
614 64:1129–1135. DOI: 10.1016/j.marpolbul.2012.03.031.

615 Mayfield AB, Fan T-Y, Chen C-S. 2013. The impacts of *ex situ* transplantation on the
616 physiology of the Taiwanese reef-building coral *Seriatopora hystrix*. *Journal of Marine*
617 *Biology* 2013:1–11. DOI: 10.1155/2013/569361.

618 McRae CJ. 2021. Exploring natural mechanisms and active enhancement of coral thermal
619 tolerance. [Unpublished PhD thesis] Simon Fraser University. Burnaby, British
620 Columbia, Canada.

621 McRae CJ, Keshavmurthy S, Meng P-J, Rosset SL, Huang W-B, Chen CA, Fan T-Y, Côté IM.
622 2022. Variable responses to chronic and acute elevated temperature of three coral species
623 from reefs with distinct thermal regimes. *Marine Biology* 169:97. DOI: 10.1007/s00227-
624 022-04071-6.

625 Meng P-J, Lee H-J, Wang J-T, Chen C-C, Lin H-J, Tew KS, Hsieh W-J. 2008. A long-term
626 survey on anthropogenic impacts to the water quality of coral reefs, southern Taiwan.
627 *Environmental Pollution* 156:67–75. DOI: 10.1016/j.envpol.2007.12.039.

628 Mieog JC, van Oppen MJH, Cantin NE, Stam WT, Olsen JL. 2007. Real-time PCR reveals a
629 high incidence of Symbiodinium clade D at low levels in four scleractinian corals across
630 the Great Barrier Reef: implications for symbiont shuffling. *Coral Reefs* 26:449–457.
631 DOI: 10.1007/s00338-007-0244-8.

632 Muscatine L. 1990. The role of symbiotic algae in carbon and energy flux in reef corals.
633 *Ecosystems of the World* 25:75–87.

634 Oakley CA, Davy SK. Cell biology of coral bleaching. *In* Coral bleaching 2018 (pp. 189-211).
635 Springer, Cham.

636 Okamoto M, Nojima S, Furushima Y, Nojima H. 2005. Evaluation of coral bleaching condition
637 *in situ* using an underwater pulse amplitude modulated fluorometer. *Fisheries Science*
638 71:847–854. DOI: 10.1111/j.1444-2906.2005.01036.x.

639 Oliver T, Palumbi S. 2009. Distributions of stress-resistant coral symbionts match environmental
640 patterns at local but not regional scales. *Marine Ecology Progress Series* 378:93–103.
641 DOI: 10.3354/meps07871.

642 Oliver TA, Palumbi SR. 2011. Many corals host thermally resistant symbionts in high-
643 temperature habitat. *Coral Reefs* 30:241–250. DOI: 10.1007/s00338-010-0696-0.

644 van Oppen MJH, Lough JM (eds.). 2018. *Coral Bleaching*. Cham: Springer International
645　　　　Publishing. DOI: 10.1007/978-3-319-75393-5.

646 Ostrander GK, Armstrong KM, Knobbe ET, Gerace D, Scully EP. 2000. Rapid transition in the
647　　　　structure of a coral reef community: The effects of coral bleaching and physical
648　　　　disturbance. *Proceedings of the National Academy of Sciences* 97:5297–5302. DOI:
649　　　　10.1073/pnas.090104897.

650 Putnam, H.M. and Edmunds, P.J., 2008, July. Responses of coral hosts and their algal symbionts
651　　　　to thermal heterogeneity. In *Proceedings of the 11th International Coral Reef
652　　　　Symposium, Fort Lauderdale, Florida* (pp. 393-397).

653 Putnam HM, Edmunds PJ, Fan T-Y. 2010. Effect of a fluctuating thermal regime on adult and
654　　　　larval reef corals: Thermal fluctuations and coral physiology. *Invertebrate Biology*
655　　　　129:199–209. DOI: 10.1111/j.1744-7410.2010.00199.x.

656 R Core Team. 2022. R: A language and environment for statistical computing. R Foundation for
657　　　　Statistical Computing, Vienna, Austria.

658 Ragni M, Airs RL, Hennige SJ, Suggett DJ, Warner ME, Geider RJ. 2010. PSII photoinhibition
659　　　　and photorepair in Symbiodinium (Pyrrhophyta) differs between thermally tolerant and
660　　　　sensitive phylotypes. *Marine Ecology Progress Series*. 406: 57-70. DOI:
661　　　　10.3354/meps08571

662 Richardson LE, Graham NAJ, Hoey AS. 2020. Coral species composition drives key ecosystem
663　　　　function on coral reefs. *Proceedings of the Royal Society B: Biological Sciences*
664　　　　287:20192214. DOI: 10.1098/rspb.2019.2214.

665 Riegl BM, Purkis SJ (eds.). 2012. *Coral Reefs of the Gulf: Adaptation to Climatic Extremes*.
666　　　　Dordrecht: Springer Netherlands. DOI: 10.1007/978-94-007-3008-3.

667 Roth MS. 2014. The engine of the reef: photobiology of the coral-algal symbiosis. *Frontiers in
668　　　　Microbiology* 5. DOI: 10.3389/fmicb.2014.00422.

669 Rouzé H, Lecellier G, Pochon X, Torda G, Berteaux-Lecellier V. 2019. Unique quantitative
670　　　　Symbiodiniaceae signature of coral colonies revealed through spatio-temporal survey in
671　　　　Moorea. *Scientific Reports* 9:7921. DOI: 10.1038/s41598-019-44017-5.

672 Safaie A, Silbiger NJ, McClanahan TR, Pawlak G, Barshis DJ, Hench JL, Rogers JS, Williams
673　　　　GJ, Davis KA. 2018. High frequency temperature variability reduces the risk of coral
674　　　　bleaching. *Nature Communications* 9:1671. DOI: 10.1038/s41467-018-04074-2.

675 Silverstein RN, Correa AMS, Baker AC. 2012. Specificity is rarely absolute in coral–algal
676　　　　symbiosis: implications for coral response to climate change. *Proceedings of the Royal
677　　　　Society B: Biological Sciences* 279:2609–2618. DOI: 10.1098/rspb.2012.0055.

678 Silverstein RN, Cunning R, Baker AC. 2015. Change in algal symbiont communities after
679　　　　bleaching, not prior heat exposure, increases heat tolerance of reef corals. *Global Change
680　　　　Biology* 21:236–249. DOI: 10.1111/gcb.12706.

681 Silverstein RN, Cunning R, Baker AC. 2017. Tenacious D: *Symbiodinium* in clade D remain in
682　　　　reef corals at both high and low temperature extremes despite impairment. *Journal of
683　　　　Experimental Biology*:jeb.148239. DOI: 10.1242/jeb.148239.

684 Smith EG, Vaughan GO, Ketchum RN, McParland D, Burt JA. 2017. Symbiont community
685   stability through severe coral bleaching in a thermally extreme lagoon. *Scientific Reports*
686   7:2428. DOI: 10.1038/s41598-017-01569-8.

687 Spalding MD, Brown BE. 2015. Warm-water coral reefs and climate change. *Science* 350:769–
688   771. DOI: 10.1126/science.aad0349.

689 Stat M, Gates RD. 2011. Clade D *Symbiodinium* in Scleractinian Corals: A "Nugget" of Hope, a
690   Selfish Opportunist, an Ominous Sign, or All of the Above? *Journal of Marine Biology*
691   2011:1–9. DOI: 10.1155/2011/730715.

692 Thomas L, Rose NH, Bay RA, López EH, Morikawa MK, Ruiz-Jones L, Palumbi SR. 2018.
693   Mechanisms of thermal tolerance in reef-building corals across a fine-grained
694   environmental mosaic: lessons from Ofu, American Samoa. *Frontiers in Marine Science*
695   4:434. DOI: 10.3389/fmars.2017.00434.

696 Thornhill DJ, Lewis AM, Wham DC, LaJeunesse TC. 2014. Host-specialist lineages dominate
697   the adaptive radiation of reef coral endosymbionts. *Evolution* 68(2): 352-67. DOI:
698   10.1111/evo.12270.

699 Turnham KE, Wham DC, Sampayo E, LaJeunesse TC. 2021. Mutualistic microalgae co-
700   diversify with reef corals that acquire symbionts during egg development. *The ISME*
701   *Journal*. 15(11): 3271-85. DOI: 10.1038/s41396-021-01007-8.

702 Ulstrup KE, Van Oppen MJH. 2003. Geographic and habitat partitioning of genetically distinct
703   zooxanthellae (*Symbiodinium*) in *Acropora* corals on the Great Barrier Reef. *Molecular*
704   *Ecology* 12:3477–3484. DOI: 10.1046/j.1365-294X.2003.01988.x.

705 Vaughan EJ, Wilson SK, Howlett SJ, Parravicini V, Williams GJ, Graham NAJ. 2021. Nitrogen
706   enrichment in macroalgae following mass coral mortality. *Coral Reefs* 40:767–776. DOI:
707   10.1007/s00338-021-02079-w.

708 Vega Thurber R, Schmeltzer ER, Grottoli AG, van Woesik R, Toonen RJ, Warner M, Dobson
709   KL, McLachlan RH, Barott K, Barshis DJ, Baumann J, Chapron L, Combosch DJ, Correa
710   AM, DeCarlo TM, Hagedorn M, Hédouin L, Hoadley K, Felis T, Ferrier-Pagès C, Kenkel
711   C, Kuffner IB, Matthews J, Medina M, Meyer C, Oster C, Price J, Putnam HM, Sawall
712   Y. 2022. Unified methods in collecting, preserving, and archiving coral bleaching and
713   restoration specimens to increase sample utility and interdisciplinary collaboration. *PeerJ*
714   10:e14176. DOI: 10.7717/peerj.14176.

715 Wang J-T, Meng P-J, Chen Y-Y, Chen CA. 2012. Determination of the thermal tolerance of
716   Symbiodinium using the activation energy for inhibiting photosystem II activity.
717   *Zoological Studies*: 6.

718 Warner M, Chilcoat G, McFarland, Fitt W. 2002. Seasonal fluctuations in the photosynthetic
719   capacity of photosystem II in symbiotic dinoflagellates in the Caribbean reef-building
720   coral *Montastraea*. *Marine Biology* 141:31–38. DOI: 10.1007/s00227-002-0807-8.

721 Winters G, Loya Y, Beer S. 2006. *In situ* measured seasonal variations in Fv/Fm of two common
722   Red Sea corals. *Coral Reefs* 25:593–598. DOI: 10.1007/s00338-006-0144-3.

723 Wyatt ASJ, Leichter JJ, Toth LT, Miyajima T, Aronson RB, Nagata T. 2020. Heat accumulation
724   on coral reefs mitigated by internal waves. *Nature Geoscience* 13:28–34. DOI:
725   10.1038/s41561-019-0486-4.

726 Yellowlees D, Rees TAV, Leggat W. 2008. Metabolic interactions between algal symbionts and
727   invertebrate hosts. *Plant, Cell & Environment* 31:679–694. DOI: 10.1111/j.1365-
728   3040.2008.01802.x.

729 Yuyama I, Nakamura T, Higuchi T, Hidaka M. 2016. Different stress tolerances of juveniles of
730   the coral *Acropora tenuis* associated with clades C1 and D *Symbiodinium*. *Zoological*
731   *Studies*. DOI: 10.6620/ZS.2016.55-19.

Figure 1

Temperature patterns from June 2018 to July 2019 at two study reefs in southern Taiwan.

Daily temperature at (A) the warmed and thermally variable reef (Outlet reef; grey) and (B) the thermally stable reef (Wanlitung reef; blue). The dashed horizontal lines show a reference temperature of 30˚C to facilitate a fast visual comparison of temperature trends between sites. (C) Daily mean temperature, (D) daily temperature range, (E) daily temperature maxima, and (F) daily temperature minima at each reef; Outlet reef (grey) and Wanlitung reef (blue). The lines are smoothed averages and shaded areas represent 95% confidence intervals. Asterisks indicate season-specific temperature difference between sites.

A. Outlet reef (warmed and thermally variable site)

B. Wanlitung reef (thermally stable site)

C. Mean daily temperature

D. Daily temperature range

E. Daily temperature maxima

F. Daily temperature minima

Summer 2018 · Fall 2018 · Winter 2019 · Spring 2019 · Summer 2019
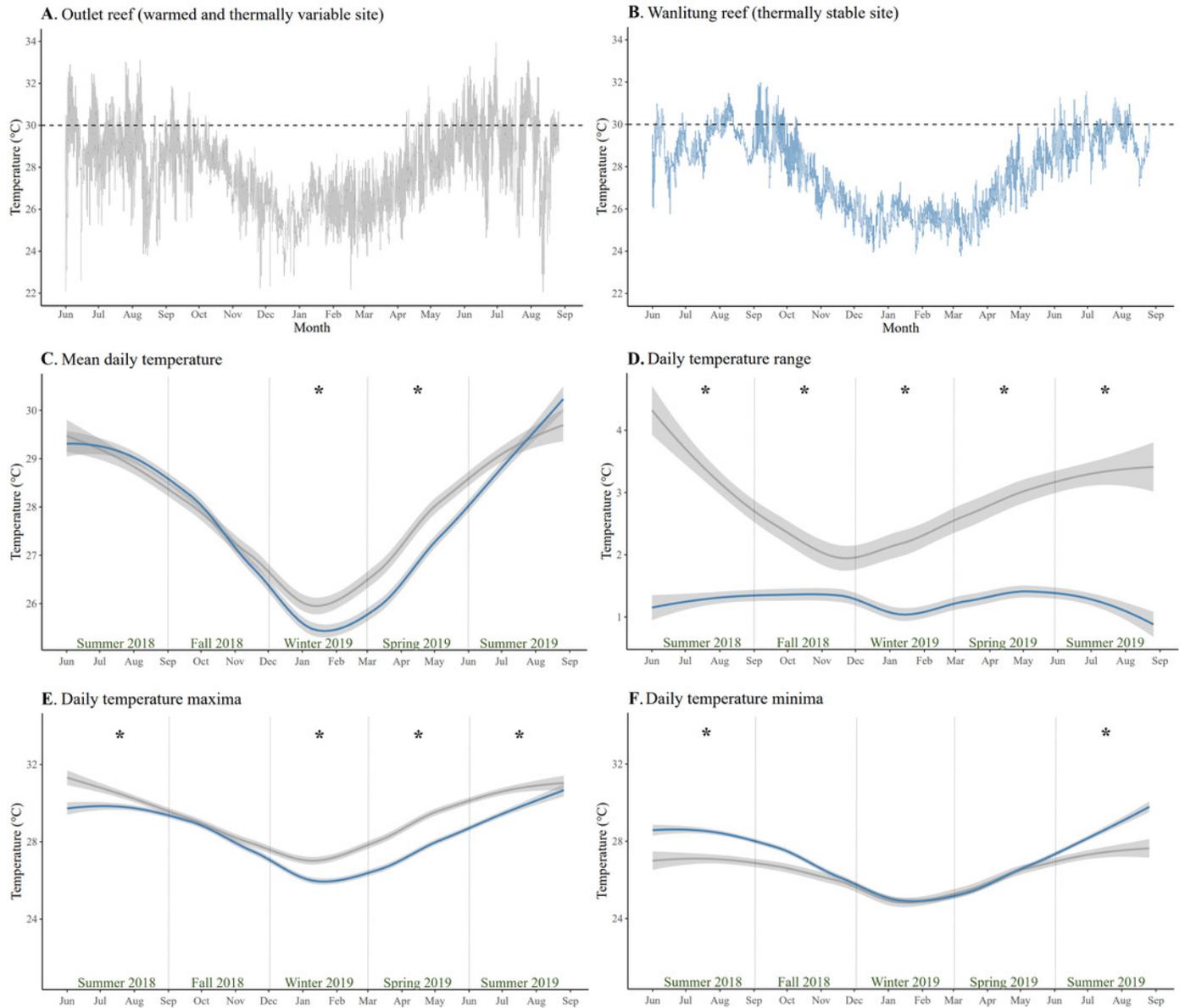
Figure 2

Detection of the presence or absence of two Symbiodiniaceae genera, *Cladocopium* (orange; C) and *Durusdinium* (black; D), in colonies of *Acropora nana*.

Colonies were monitored seasonally at a warmed and thermally variable reef (Outlet reef; panel A) and a thermally stable reef (Wanlitung reef; panel B) in southern Taiwan, from summer 2018 to spring 2019. Summer 2019 samples were not analyzed due to COVID-19 laboratory access limitations. Symbols indicate individual colony data (e.g., in panel A squares show the data for colony #OT_AN5 whereby *Cladocopium* detection is shown in orange and *Durusdinium* detection is shown in black).
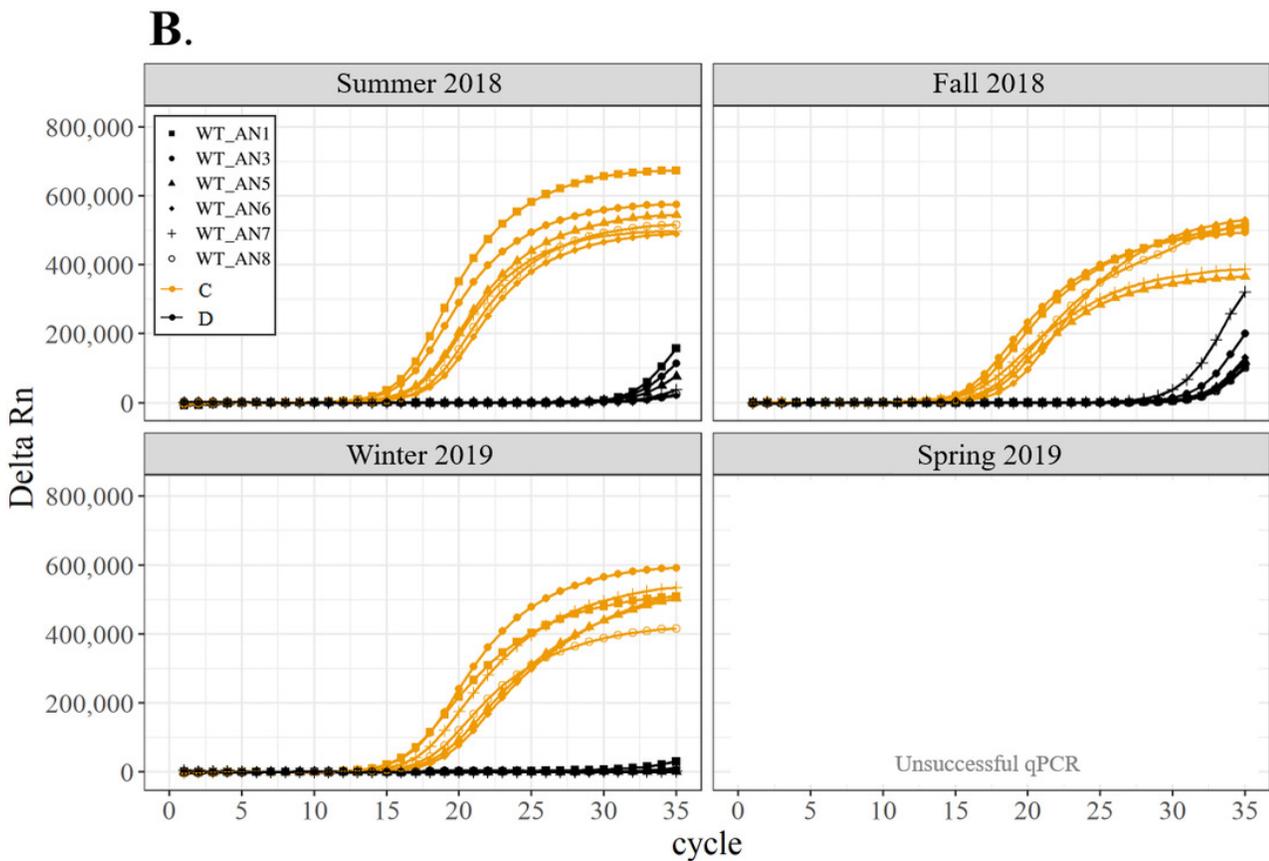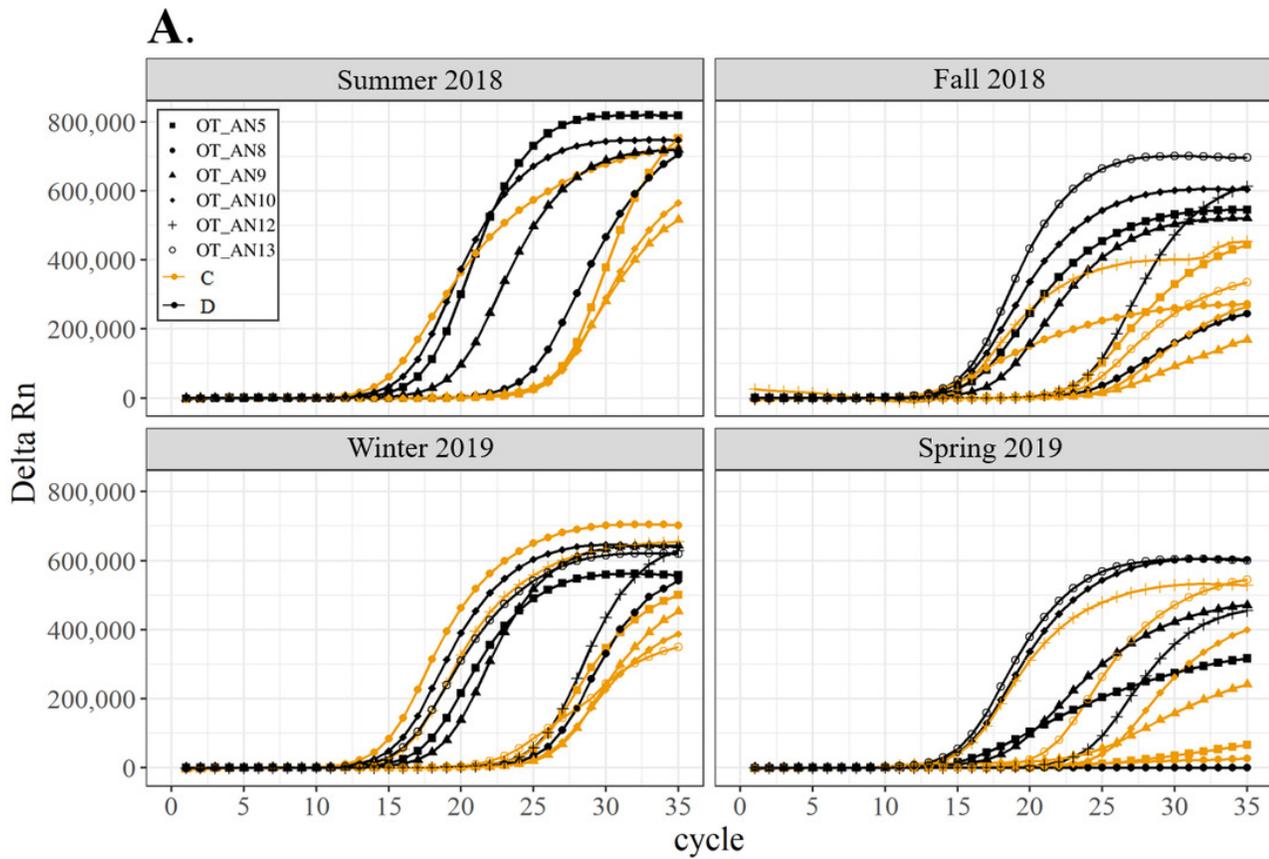
Figure 3

Detection of the presence or absence of two Symbiodiniaceae genera, *Cladocopium* (orange; C) and *Durusdinium* (black; D), in colonies of *Pocillopora acuta*.

Detection of the presence or absence of two Symbiodiniaceae genera, *Cladocopium* (orange; C) and *Durusdinium* (black; D), in colonies of *Pocillopora acuta*. Colonies were monitored seasonally at a warmed and thermally variable reef (Outlet reef; panel A) and a thermally stable reef (Wanlitung reef; panel B) in southern Taiwan, from summer 2018 to spring 2019. Summer 2019 samples were not analyzed due to COVID-19 laboratory access limitations. Symbols indicate individual colony data (e.g., in panel A squares show the data for colony #OT_PA1 whereby *Cladocopium* detection is shown in orange and *Durusdinium* detection is shown in black).
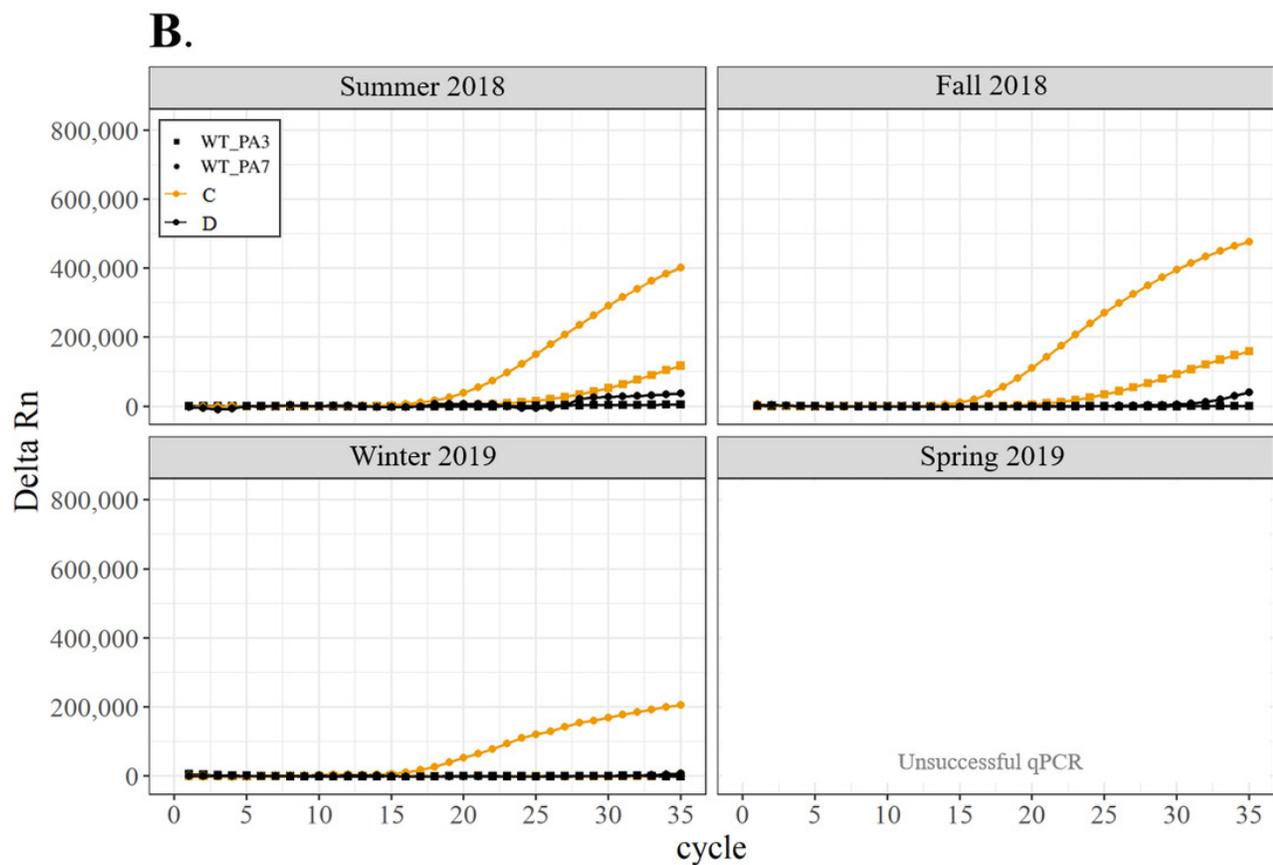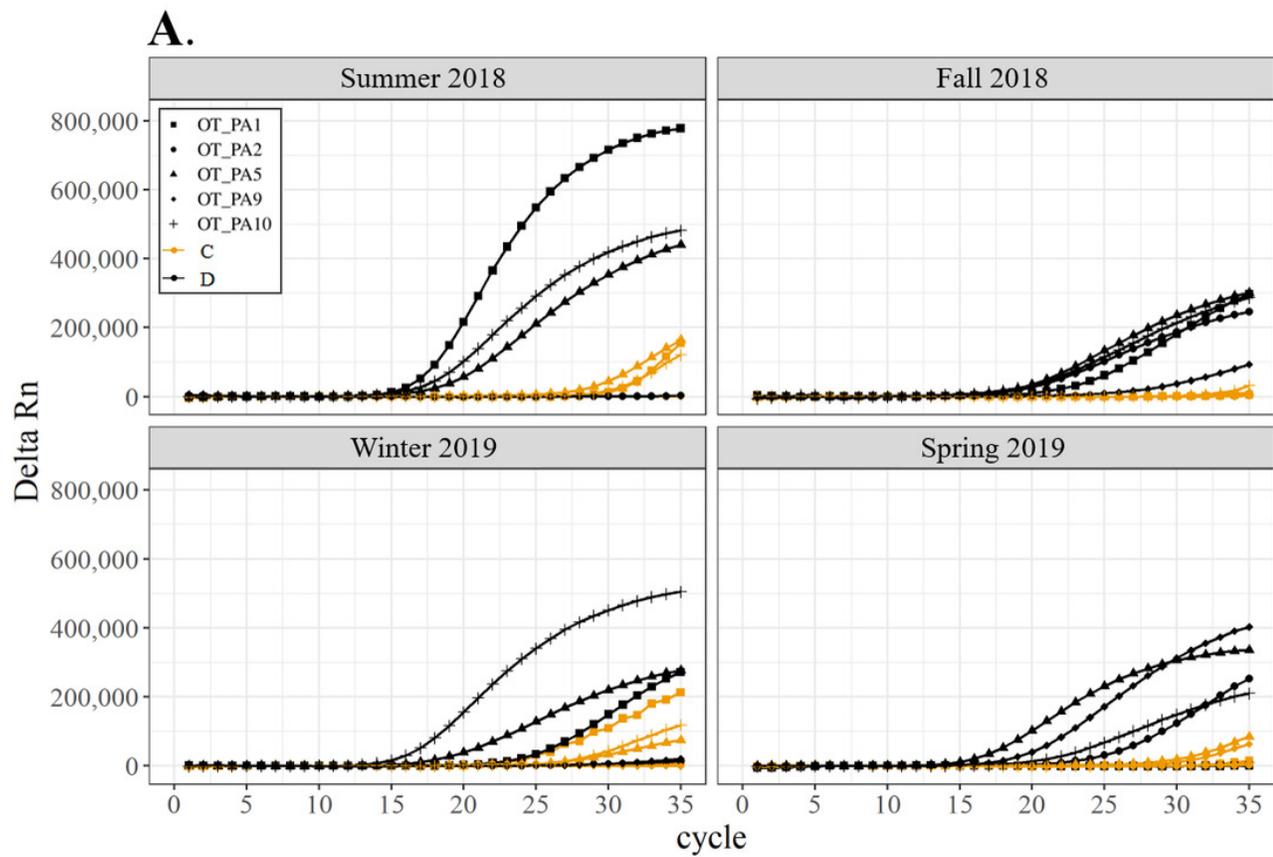
Figure 4

Detection of the presence or absence of two Symbiodiniaceae genera, *Cladocopium* (orange; C) and *Durusdinium* (black; D), in colonies of *Porites lutea*.

Colonies were monitored seasonally at a warmed and thermally variable reef (Outlet reef; panel A) and a thermally stable reef (Wanlitung reef; panel B) in southern Taiwan, from summer 2018 to spring 2019. Summer 2019 samples were not analyzed due to COVID-19 laboratory access limitations. Symbols indicate individual colony data (e.g., in panel A squares show the data for colony #OT_PL1 whereby *Cladocopium* detection is shown in orange and *Durusdinium* detection is shown in black).
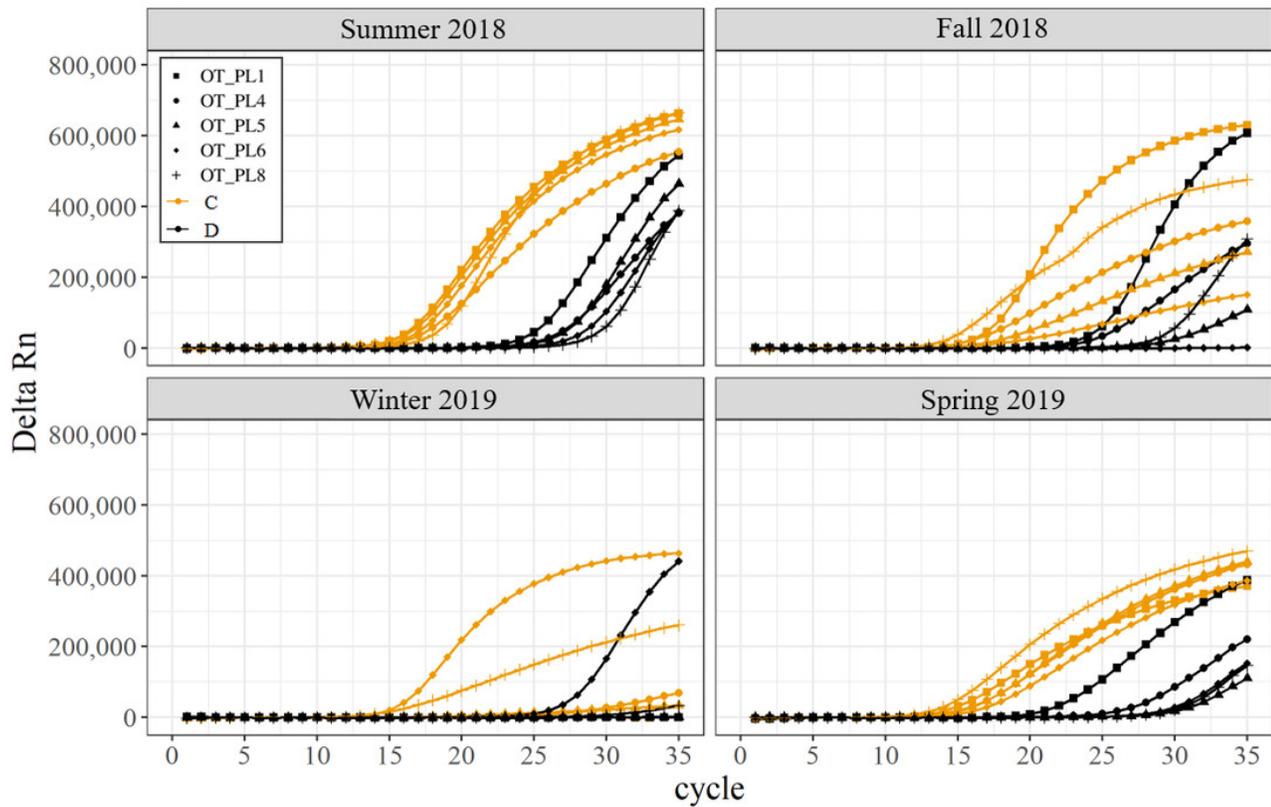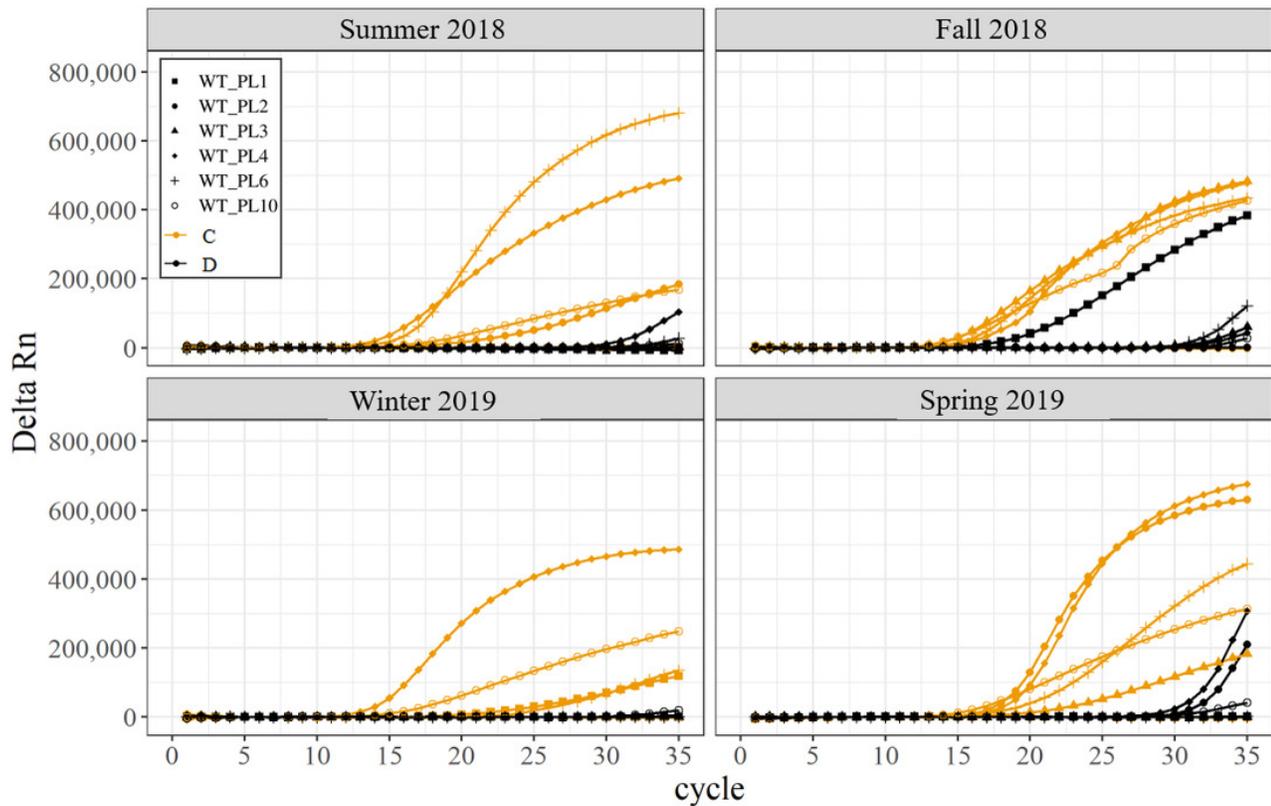
Figure 5

Maximum quantum yield of photosystem II (F_v/F_m) of algal symbionts hosted by three coral species.

Colonies of *Acropora nana* (A), *Pocillopora acuta* (B), and *Porites lutea* (C) were monitored at a warmed and thermally variable site, Outlet reef (OT; grey), and a thermally stable site, Wanlitung reef (WT; blue) in southern Taiwan, from summer 2018 to summer 2019. Asterisks indicate significant reef site differences, which was assessed seasonally and independently for each species. Coloured letters indicate significant differences across seasons independently for colonies at Outlet reef (grey letters) and Wanlitung reef (blue letters) separately assessed for each coral species.

A. *Acropora nana*

B. *Pocillopora acuta*

C. *Porites lutea*