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High physiological function for corals with thermally tolerant, host-adapted symbionts

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The flexibility to associate with more than one symbiont may considerably expand a host's niche breadth. Coral animals and dinoflagellate microalgae represent one of the most functionally integrated and widespread mutualisms between two eukaryotic partners. Symbiont identity greatly affects a coral's ability to cope with extremes in temperature and light. Over its broad distribution across the Eastern Pacific, the ecologically dominant branching coral, *Pocillopora grandis*, depends on mutualisms with the dinoflagellates *Durusdinium glynnii* and *Cladocopium latusorum*. Measurements of skeletal growth, calcification rates, total mass increase, calyx dimensions, reproductive output and response to thermal stress were used to assess the functional performance of these partner combinations. The results show both host–symbiont combinations displayed similar phenotypes; however, significant functional differences emerged when exposed to increased temperatures. Negligible physiological differences in colonies hosting the more thermally tolerant *D. glynnii* refute the prevailing view that these mutualisms have considerable growth tradeoffs. Well beyond the Eastern Pacific, pocilloporid colonies with *D. glynnii* are found across the Pacific in warm, environmentally variable, near shore lagoonal habitats. While rising ocean temperatures threaten the persistence of contemporary coral reefs, lessons from the Eastern Pacific indicate that co-evolved thermally tolerant host–symbiont combinations are likely to expand ecologically and spread geographically to dominate reef ecosystems in the future.

1. Introduction

Symbioses profoundly influence the diversity, ecology, and evolution of life on Earth. In particular, mutualisms function through reciprocal exploitation that ultimately provides net benefits to each partner, with the resulting functionality of the unit constrained to the attributes of each partner. Reef-building corals depend on nutrients translocated from endosymbiotic photosynthetic dinoflagellates in the family Symbiodiniaceae for survival

and growth. Numerous different host–symbiont combinations exhibit a wide breadth of functional diversity important to the persistence and resiliency of the ecosystems they construct [1–3]. Notably, the sensitivity of reef-building corals to acute environmental stressors including prolonged and/or extreme periods of irradiance and/or temperature stress is influenced by the identity and physiology of their dinoflagellate partner [2,4–7]. Corals hosting symbionts that maintain physiological function under such conditions have reduced symbiont cell loss (coral bleaching), less mortality, and faster recoveries [1,8–11]. A body of evidence suggests that the costs of thermal tolerance manifest as reduced nutrient translocation [12–15] and significant negative physiological tradeoffs to the host coral such as reduced growth and fecundity [16–18], although some findings contradict this perception [19–23].

While partner specificity is largely intrinsic to most coral–dinoflagellate mutualisms, the fidelity between host and symbiont is partially influenced by prevailing environmental conditions, and why thermal history and light availability often explains the dominance of certain host-compatible symbionts over others [24,25]. Partner specificity is especially magnified in coral taxa where symbionts are transferred during egg maturation (vertical acquisition or transmission) [26,27]. Contrary to horizontal transmission (symbiont acquired from the environment), vertical transmission reinforces the maintenance of certain host–symbiont combinations for generations, leading to ecological (host habitat) specialization by the symbiont [28]. Yet, vertical transmission only occurs in several, albeit widespread and ecologically successful, host taxa including members of the genera *Porites* and *Montipora*, as well as corals in the family Pocilloporidae. In some cases, these co-evolved host–symbiont pairings may have greater functional integration compared to associations reliant on horizontal transmission [22].

The first reports that related differential colony mortality to the identity of the resident symbiont originated in the Tropical Eastern Pacific (TEP) following the 1997–1998 El Niño Southern Oscillation (ENSO) event when water temperatures were 2–4°C warmer than historical average temperatures [1]. *Pocillopora grandis* colonies that hosted *Durusdinium glynnii* remained pigmented during this thermal anomaly and experienced little to no mortality, while colonies that bleached contained *Cladocopium latusorum* as did colonies of *P. verrucosa* (presumably) with *Cladocopium pacificum* [1,29]. This differential response to environmental stressors was later confirmed when 90% of *P. grandis* colonies containing *C. latusorum* and just 10% of colonies with *D. glynnii* visibly bleached during cold-water events in 2007 and 2008 [30,31]. Thus, the greater stress tolerance of colonies with *D. glynnii* mostly explains the relative dominance of this host–symbiont combination across the Eastern Pacific tropical and sub-tropical coasts of North and Central America [1,9,32,33].

The Eastern Pacific is an ideal study system to compare how different symbionts can affect the functionality of an ecologically dominant coral. *Pocillopora grandis* with either *D. glynnii* or *C. latusorum*, occur at similar depths and in many of the same habitats, with few colonies containing mixtures of each symbiont [9,34,35]. Here, the functional performance of both partnerships was studied under normal conditions and during a thermal stress experiment. Critical attributes of these mutualisms, including symbiont cell densities and division rates, photophysiology, colony growth, calcification, calyx dimensions and fecundity were examined to evaluate how different symbiont species affect the host animal's well-being and reveal possible metabolic trade-offs associated with thermal tolerance.

2. Methods

(a) Colony sampling and transect configuration for symbiont species identification, ecological prevalence and within-host abundance

Three independent sampling methods were conducted to sample colonies of *Pocillopora grandis*, also referred to in the literature using the junior synonym *P. eydouxi*. This is the only genetically verified *Pocillopora* species found along Mexico's Pacific coastline [36]. In 2004, during an initial biodiversity survey, 129 colonies were sampled using a hammer and chisel from various habitats at depths of 1–8 m in the Gulf of California region around La Paz. At Punta Galeras Reef (24° 21.2567 N, 110° 17.0833 W) and La Gaviota Island (24° 17.2 N; 110° 20.3333 W) three 25 m long linear permanent transects were established at each location in May 2006 as described in LaJeunesse *et al.*, [34] where 18–24 tagged colonies were sampled per transect (122 total). Lastly, three 20 m diameter circular/polar plot surveys (greater than 30 m apart) at Punta Galeras Reef and three at La Gaviota Island were conducted to randomly sample colonies in a circular area according to Baums *et al.* [37] (118 colonies total) [37]. Symbiont species were identified by DGGE-ITS2/ITS1 profiling and sequencing as described in [27,34,38]. Each sample used was sequenced following each field season-sampling/experimental timepoint- providing a total of 595 of samples. The symbiont identity in colonies used for all experiments was confirmed using the same methods.

(b) Symbiont cell sizes, mitotic indices, and densities

Tissue was removed from branch fragments representing each of seven colonies containing *C. latusorum* and nine colonies with *D. glynnii* in July 2007 (colonies were from the established transects with known symbiont species). One ml of tissue slurry was preserved with 10uL of 10% glutaraldehyde. Symbiont cell sizes were measured at 400× on an Olympus Bx61 compound microscope (Olympus Corp., Tokyo, Japan) with the ORCA ER (Model C4742-80) and Olympus DP71 at the Penn State Microscopy Facility. The maximum length and width of at least 50 cells per colony were measured using ImageJ. Average ellipsoid cell volume was calculated using the formula,

$$V = \frac{4}{3}\Pi\, abc$$

where a, b, and c is equal to half the length, width and height (equal to width), respectively.

Maximum cell division time was estimated by collecting cells every three hours from 03.30 to 21.30 from polyps 1.5–2 cm below the branch tips of three different *Pocillopora* colonies (genets) containing *D. glynnii* and three colonies containing *C. latusorum* in July 2007. Symbiont cells were isolated from host tissues with a pipette tip to remove and macerate 1–2 polyps. The slurry was placed in a 0.5 ml Eppendorf tube and fixed with 1% glutaraldehyde. Cell number and dividing cells (recorded as doublets and tetrads) were recorded with a haemocytometer (the cells in each of the three 10 µl sub-samples were counted). The mitotic index was calculated by dividing the number of dividing cells by the total cells present [39].

Symbiont densities were estimated by removing tissue from 8 coral fragments (four fragments each for *C. latusorum* and *D. glynnii*) with a WaterPik and filtered seawater (0.4 µm) in July 2007 [40]. The tissue slurry volume was recorded, and symbiont cells were counted using a haemocytometer on a light microscope. The hot wax method [41] was used to measure coral surface area, as described in detail in [9]. Total symbiont-to-host biomass ratios were calculated by multiplying the average symbiont cell volume by the average symbiont cell densities per host tissue area.

(c) Relative colony fecundity

During July 2008, nearing the first peak in *Pocillopora grandis* spawning in the Gulf of California [42], three fragments each from six tagged colonies with *D. glynnii* and seven colonies with *C. latusorum* were collected using a hammer and chisel and preserved in 10% seawater formalin. Fragments were then decalcified in 10% hydrochloric acid for 24 h and embedded in paraffin wax. Coral tissue was sectioned (10 µm) with a microtome, and six slides were used per fragment with each containing three to five sections approximately 0.5 cm below the growing tip and three to five sections approximately 0.5 cm above the base of the fragment. Slide images were recorded on an Olympus Bx61 microscope (Olympus Corp., Tokyo, Japan) with the ORCA ER (Model C4742-80) and Olympus DP71 at the Penn State Microscopy Facility. Oocytes with a visible, stained nucleus (indicating the centre and the maximum diameter of the oocyte) were photographed, and the maximum Ferret diameter of each oocyte was measured using ImageJ. Separately, the number of oocytes and spermaries from three randomly selected, cross-sectional polyps per slide were counted and used as a proxy for relative fecundity.

(d) Host growth, calcification, linear extension and calyx dimensions

One fragment per colony from 6 colonies containing *C. latusorum* and 5 colonies with *D. glynnii* were collected in May 2010 and maintained in outdoor seawater flow-through tables at 26°C under a shade cloth providing a maximal mid-day irradiance of approximately 400 µmol quanta $m^{-2} 2 s^{-1}$. Instantaneous rates of calcification were measured following the procedure from Yao and Byrne 1998 [43] which is based on the alkalinity anomaly technique wherein each molar equivalent reduction in total alkalinity of seawater corresponds to one precipitated mole of $CaCO_3$ [44,45]. Each fragment was incubated for one hour in 200 ml acrylic chambers containing 0.45 µm filtered seawater with a stir bar and held at a constant temperature of 25.5°C ± 0.5 with 400 µm quanta $m^{-2}s^{-1}$ from 6W pure white LED bulbs. The instantaneous calcification rate was calculated by spectrophotometrically measuring the change in total alkalinity of seawater of the incubation seawater using an Ocean Optics USB4000 spectrophotometer (Ocean Insight, Orlando Fl.).

In June 2008, coral growth was recorded by buoyant weight [46] by collecting and initially weighing fragments from 33 colonies containing *D. glynnii* and 44 colonies containing *C. latusorum*. Fragments were mounted on 1.5″ PVC couplers and were subsequently attached to the reef and then collected and re-weighed four months later in October 2008 and one year later in June 2009. DNA was extracted from each fragment and analysed to identify symbiont species after the last weight measurement.

Coral branch linear extension was determined *in situ* over the course of eight months from October 2008 to June 2009. A plastic band was placed on four randomly selected branches approximately 50 cm near the centre of 10 colonies containing *D. glynnii* and 10 colonies containing *C. latusorum*, and the distance from the band to the branch tip was measured. The band-to-branch length was again measured in June 2009 for all except 5 *D. glynnii* colonies, which were removed from the analysis as half of the banded branches were lost from the colony. DNA was extracted and analysed to identify symbiont species at the end of the experiment.

Five calices were randomly selected for measurement from five previously collected, genotyped, and photographed colonies containing *C. latusorum* and 15 containing *D. glynnii*. The maximum and minimum diameters of each calyx cup and the distance to the nearest calyx were measured using ImageJ.

(e) Experimental thermal stress and photophysiology

As described in detail in [31], tagged *P. grandis* colonies containing *C. latusorum* and *D. glynnii* were collected from 3–5 m and brought back into shaded seawater flow-through tanks at Universidad Autónoma de Baja California Sur in July 2007, which received a similar maximal mid-day irradiance as that recorded on the reef (approx. 950–1000 µmol photons $m^{-2} s^{-1}$). Twelve host fragments per symbiont species were mounted using marine epoxy onto 1.5″ PVC couplers, divided and placed into control tanks, and maintained at 26°C for 36 h for a short acclimation period. In the experimental tanks, temperature was increasingly ramped 1.5°C per day until reaching 32°C and then held at 32°C for 7 more days, while control tanks remained at 26°C for the entirety of the experiment. During acclimation and throughout the experiment, single-turnover active chlorophyll a fluorescence was monitored with a fluorescence induction and relaxation (FIRe) fluorometer fitted with a fibre optic probe (Satlantic). After dark acclimating fragments for 20 min, a 120 µs saturation light pulse was applied. Fluorescence kinetics were averaged from ten fluorescence induction curves ($n = 10$) and were fit to a biophysical model [47] in order to calculate the maximum quantum yield of photosystem II, F_v/F_m, as $(F_m - F_o)/F_m = F_v/F_m$), as well as the PSII functional absorption cross-section (σ_{PSII}) which is a measure of photon capture efficiency and is indicative of the ability to absorb and use light, and the reoxidation rate of photosystem II (PSII) (τ), which indicates the speed at which electrons are moving between primary and secondary quinones in PSII. Decreased photosynthetic efficiency, slowed reoxidation rates, and larger σ_{PSII} are often observed with thermal/light stress [6,22,23,49,48]. After 24 h at either control (26°C) or treatment (32°C), symbiont cells were isolated and the mitotic index was quantified during maximum cell division.

(f) Data analysis

All data were checked for normality using the Shapiro–Wilk test and qqplots. Where the assumption of normality was not met, non-parametric tests were used to test deviations from null hypotheses. During the high-temperature experiment, F_v/F_m, σ_{PSII}, τ, and mitotic indices were compared using the linear mixed-effects models (LMMs) using the lme4 package [50], with fixed effects including symbiont species, day, and treatment, and random effect of the fragment, to assess the influence of symbiont species and thermal exposure (28° or 32°C) and duration (1–7 days) on a colony's photophysiology. Pairwise *post hoc* comparisons were performed using the 'emmeans' package [51]. Cell densities were normalized to a regression-based standard curve of host tissue surface areas and total densities and cell sizes were compared using Student's *t*-test. Buoyant weights were tested with LMMs with fixed effects of symbiont species, time, and random effect of the fragment, with post hoc comparisons as previously described. Instantaneous calcification rates were analysed using Student's *t*-test. The non-parametric Wilcoxon rank sum test was used to analyse branch linear extension, oocyte size, relative fecundity, as well as calyx diameters and distances to the nearest neighbour calyx. Metric means and standard deviations are

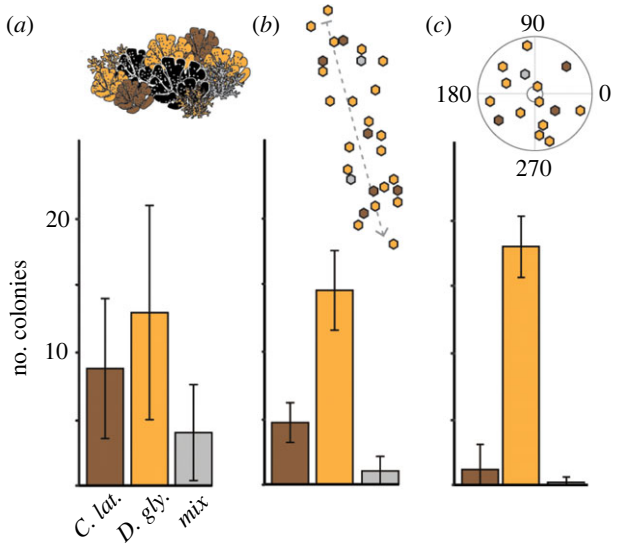

Figure 1. *Pocillopora grandis* colonies in the Eastern Pacific predominately host *Durusdinium glynnii*, with some colonies hosting *Cladocopium latusorum* and even fewer with mixtures of both from three independent sampling approaches: (*a*) Sampling for symbiont biodiversity in the region around La Paz, Mexico, conducted in 2004 ($n = 129$). (*b*) Samples from tagged colonies along 25 m length linear transects established at Punta Galeras and Isla Gaviota in 2006 (>18 colonies per transect, $n = 122$ colonies from a total of six transects, three at each location). (*c*) Randomized sampling of colonies from 20 m diameter circular plots in 2009 ($n = 118$). Each point represents the number of colonies with the corresponding symbiont per site. Brown indicates the proportion of colonies with *C. latusorum* while yellow indicates those with *D. glynnii* as the dominant symbiont.

reported. For each test, outliers were removed and re-analysed, with deviations from the interpretations noted.

3. Results

(a) High prevalence of colonies housing thermally tolerant *D. glynnii* and rare detection of symbiont mixtures.

Using three different sampling approaches, *D. glynnii* was most prevalent in a majority of *P. grandis* colonies (figure 1*a–c*). Differences in the prevalence of colonies with *C. latusorum* ranged from 6–34% which differed from location to location, mode of sampling, and year. Colonies with mixtures of each symbiont were typically rare (≤5%), but highest in areas where colonies with *C. latusorum* were most common (approx. 15% of colonies; figure 1*b*). The co-occurrence of both host–symbiont pairing allowed for the comparison of their phenotypic and physiological attributes (figure 2*a*).

(b) Total volumes of the symbiont populations were generally equivalent for each host–symbiont combination

The mean cell sizes (width and length) of *D. glynnii* were smaller than *C. latusorum* (figure 2*b*). The mean cell volumes calculated for *D. glynnii* were therefore significantly smaller than *C. latusorum* ($p < 0.001$) (table 1). Mean *D. glynnii* cell densities were greater than the cell densities of *C. latusorum* ($p = 0.006$; figure 2*c*; table 1). The marked difference in cell volume when combined with cell density differences in the host, results in equivalent average symbiont volume per area of host tissue; 18.61 for colonies with *D. glynnii* and 18.41 with *C. latusorum*.

(c) Cell division rates differed diurnally and seasonally

Over a 24-h sampling cycle in July, cell division rates for both species were highest 1–3 h after sunrise (figure 2*d*). The same peak division time was confirmed in January (data not shown). This diurnal peak in cell division (mitotic index) was twice as high (6% versus 3%) for *D. glynnii* than *C. latusorum* in the summer (July) (figure 2*d*). However, in the winter, the peak mitotic index increased significantly for *C. latusorum* while it was significantly decreased for *D. glynnii* to the extent that peak per cent cell division was higher for *C. latusorum* than *D. glynnii* (5% versus 3%; $p < 0.001$f; figure 3*d*; table 2).

(d) Oocyte size, not fecundity, differed among host–symbiont pairings

Fecundity (figure 2*e*), estimated as the average number of gametes (oocytes and spermaries) per polyp, was similar between the superior (approx. 2 cm from tip) and inferior (6–8 cm from tip) positions of each coral branch examined regardless of the symbiont hosted ($p > 0.31$; table 1). Moreover, fecundity was similar between each host–symbiont combination ($p > 0.1$; figure 2*f*; table 1). Oocyte size ranges followed a bimodal distribution in all colonies (figure 2*g*), corresponding to different early stages in oocyte development (i.e. I–III) as well as for mature oocytes (stage IV). Colonies with *C. latusorum* had larger oocytes than colonies with *D. glynnii* ($p < 0.001$; figure 2*g*; table 1).

(e) Biomass increase, calcification rates, linear extension as well as calyx cup sizes and distance of separation were equivalent between host–symbiont pairings

Mean buoyant weights of small out-planted experimental colonies increased 40% after 6 months and 260% after one year ($p < 0.001$). There was no statistical difference in weight gain between colonies with *D. glynnii* or *C. latusorum* ($p > 0.93$; figure 2*h*; table 1). Likewise, branches with either *D. glynnii* or *C. latusorum* calcified at the same rate ($p = 0.12$; figure 2*i*; table 1) and branch linear extension of large adult colonies *in situ* was similar after eight months ($p = 0.17$; figure 2*j*; table 1). Average calyx sizes between colonies with *D. glynnii* or *C. latusorum* were also similar ($p = 0.053$) as was the distance between nearest calices ($p = 0.78$; table 1; electronic supplementary material, figure S1).

(f) Colonies with *D. glynnii* tolerated high temperatures, while colonies with *C. latusorum* underwent photoinactivation

No photophysiological differences were observed in the maximum quantum yield of photosystem II (F_v/F_m), functional absorption cross section (σ_{PSII}) or rate of reoxidation (τ) in control or heat treatment colonies hosting *D. glynnii* ($p > 0.2$; figure 3*a*,*b*; table 2). These variables did not change in *C. latusorum* control colonies besides having slower reoxidation rates (τ) by day 6 of the experiment ($p < 0.004$; table 2). However, F_v/F_m declined significantly in heat-treated *C. latusorum* colonies beginning on day 3 of the experiment ($p = 0.01$; table 2) and continued until day 7 ($p < 0.001$; table 2). The σ_{PSII} increased by day 3

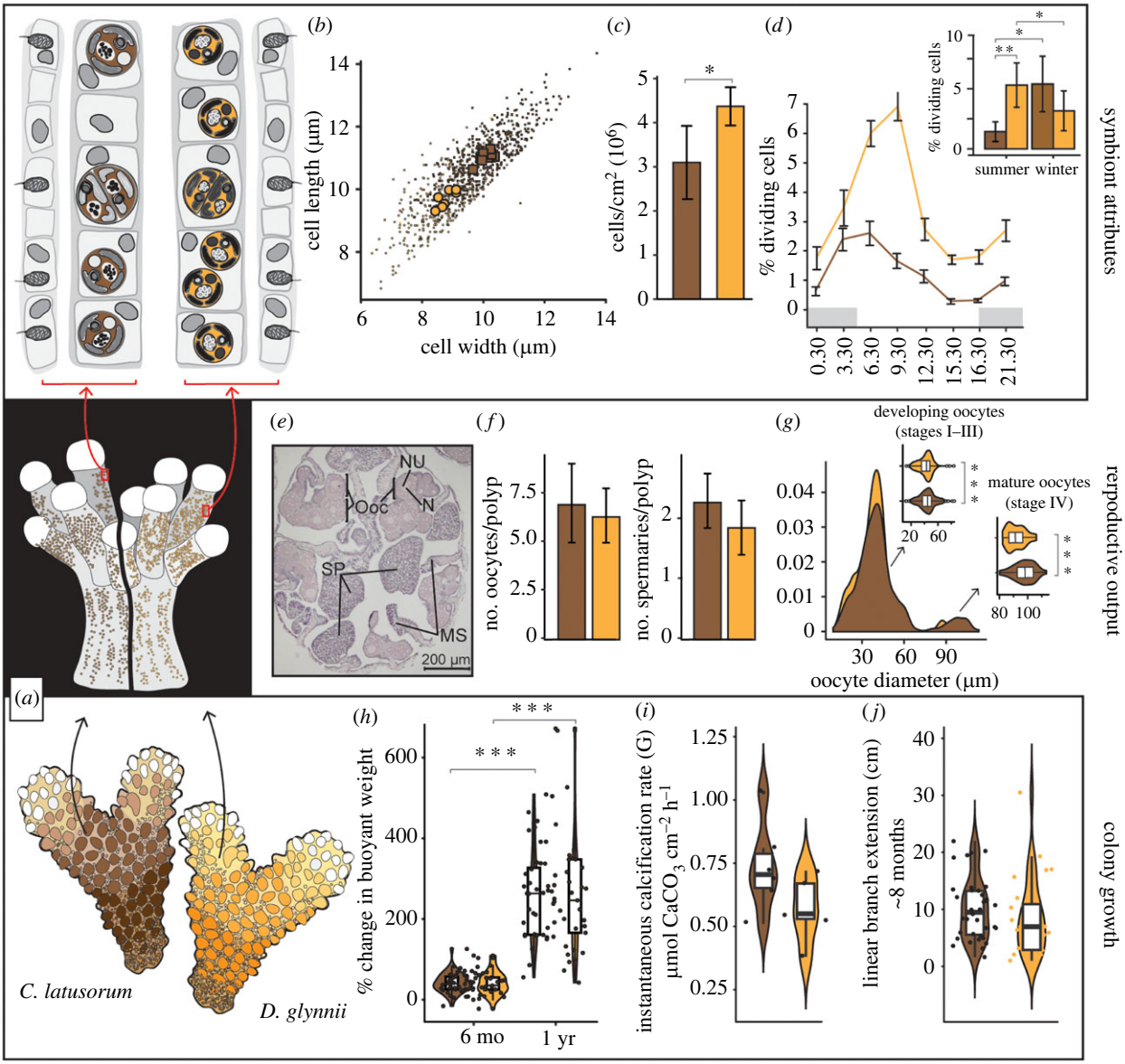

Figure 2. Emergent properties of co-evolved host–symbiont mutualisms. (*a*) Analyses of two host–symbiont combinations conducted at different biological scales ranging from cellular dynamics of the dinoflagellate symbiont (top), fecundity at the polyp scale (middle), to colony-scale growth rates (bottom). Colonies of *Pocillopora grandis* dominated by *Durusdinium glynnii* appear yellow-orange, while colonies with *Cladocopium latusorum* appear brownish. (*b*) Cell dimensions (length and width) of *C. latusorum* are larger than those of *D. glynnii* (large symbols represent mean dimensions of cells obtained from independent colonies), (*c*) Cell densities in host tissues were different for colonies with *C. latusorum* (brown) or *D. glynnii* (yellow-orange) ($p = 0.006$). (*d*) Each symbiont exhibited diurnal oscillation in mitotic indices with peak division rates 1–3 h after sunrise (grey shading = night). Summer-time cell division rates (proportion of dividing cells to the total number of cells) measured over a 24-hour period for *C. latusorum* and *D. glynnii*. Inset shows significant differences in peak mitotic indices in the summer (S) versus the winter season (W) for each symbiont species. (*e*) Histological transverse cross-section of polyp showing mesenteries (M), oocytes (Ooc), oocyte nuclei (N), oocyte nucleolus (NU), and spermaries (SP). (*f*) Average number of oocytes (left, $p = 0.52$) and spermaries (right, $p = 0.1$) per polyp for *P. grandis* with *C. latusorum* or *D. glynnii*, respectively. (*g*) Bi-modal size distribution of developing (stages I–III) and mature (stage IV) oocytes. Oocytes in colonies with *D. glynnii* were smaller for each developmental stage ($p < 0.001$). (*h*) Increases in buoyant weights at 6 months and one year ($p > 0.93$), (*i*) instantaneous calcification rates ($p = 0.12$), and (*j*) linear branch extension ($p = 0.17$) were similar between colonies with each symbiont species. Error bars represent one standard deviation.

($p = 0.02$; table 2) and day 7 ($p < 0.001$; table 2) in heat-treated *C. latusorum* compared to controls, and the reoxidation rate slowed at day 7 ($p = 0.002$; figure 3; table 2).

(g) Cell division rates of *D. glynnii* increased at high experimental temperatures

In experimentally heat-treated colonies, the mitotic indices of *D. glynnii* were significantly higher than the controls ($p < 0.001$; figure 3*c*; table 2), whereas peak mitotic indices of *C. latusorum* were similar to the controls during heat treatment ($p = 0.82$; figure 3*c*; table 2).

4. Discussion

(a) The functional convergence of different host–symbiont combinations

(i) Mutualisms converged on a functionally stable and productive unit

The similarities in growth as well as gamete production, seen here, indicate that colonies derive similar metabolic benefits from hosting evolutionarily divergent symbionts (figure 2). The steady-state condition, or phenotype, of *P. grandis* with each symbiont species is noticeably distinct. Yet the emergent

Table 1. Summary statistics for symbiont cell size, volume, mitotic index (MI) and density as well as host relative fecundity and growth measurements between *Cladocopium latusorum* and *Durusdinium glynnii*. Values represent mean ± ci. Significant *p*-values ($p < 0.05$) are in bold.

	Cladocopium latusorum	*Durusdinium glynnii*	*p*-value
symbiont cell length (µm)	**11.02 ± 0.08**	**9.68 ± 0.09**	**<0.001**
symbiont cell width (µm)	**10.08 ± 0.08**	**8.77 ± 0.09**	**<0.001**
symbiont cell volume (μm^3)	**594.76 ± 14.2**	**425.86 ± 12.49**	**<0.001**
symbiont cell densities ($cells^{-1}$ cm^2)	**3 095 985 ± 829 596**	**4 369 204 ± 433 237**	**0.006**
symbiont volume per host area	**7714.3 ± 2029**	**12039.7 ± 3957**	**0.005**
symbiont summer MI maximum	**1.42 ± 0.28**	**7.82 ± 1.33**	**<0.001**
symbiont winter MI maximum	5.4 ± 1.47	3.17 ± 1.05	0.066
symbiont MI during summer heated treatment	0.45 ± 0.22	11.58 ± 2.59	0.004
symbiont MI during summer control treatment	1.18 ± 0.45	4.49 ± 0.56	0.26
F_v/F_m in thermal treatment (experiment day 7)	**0.35 ± 0.081**	**0.46 ± 0.026**	**<0.001**
F_v/F_m in control treatment (experiment day 7)	0.46 ± 0.029	0.47 ± 0.044	0.68
σ_{PSII} in thermal treatment (experiment day 7) ($Å^2$)	**320 ± 33.23**	**255 ± 19.24**	**<0.001**
σ_{PSII} in control treatment (experiment day 7) ($Å^2$)	248 ± 32.98	242 ± 34.71	0.62
τ in thermal treatment (experiment day 7) (µseconds)	**600 ± 78.91**	**425 ± 99.63**	**0.002**
τ in control treatment (experiment day 7) **(µseconds)**	457 ± 81.01	397 ± 104.43	0.18
number of oocytes per polyp	6.96 ± 2.03	6.32 ± 1.41	0.52
number of spermaries per polyp	2.28 ± 0.44	1.84 ± 0.47	0.10
size of developing oocytes (µm)	**38.44 ± 0.81**	**35.82 ± 0.61**	**<0.001**
size of mature oocytes (µm)	**96.7 ± 2.13**	**91.31 ± 2.06**	**<0.001**
% change in buoyant weight: 6 months	41.55 ± 14.2	40.68 ± 16.4	0.97
% change in buoyant weight: 1 year	262.03 ± 14.2	263.88 ± 16.3	0.093
instantaneous calcification rate (µmol $CaCO_3$ cm^2 h^{-1})	0.73 ± 0.18	0.57 ± 0.16	0.12
linear extension (cm)	10.02 ± 1.81	8.70 ± 2.74	0.17
calyx max diameter (cm)	0.95 ± 0.057	0.89 ± 0.034	0.094
calyx min diameter (cm)	0.78 ± 0.052	0.72 ± 0.03	0.053
distance to nearest calyx (cm)	0.26 ± 0.046	0.25 ± 0.019	0.78

effect of each combination produces functionally similar mutualisms under normal environmental conditions. While colonies with *C. latusorum* have considerably fewer symbiont cells per surface area, this difference is compensated by the greater *C. latusorum* cell size relative to *D. glynnii* (figure 2*b*). Therefore, the total standing biomass of each resident symbiont population is nearly equivalent and partially explains the similarities in attributes related to colony growth and reproduction. While estimates of nutrient translocation were beyond the scope of this study, independent research recently determined that colonies with *D. glynnii* received considerable nutrient inputs from this symbiont [23]. Taken together, these findings emphasize high functionality in different mutualisms, especially in ones that are co-evolved [22,23].

There was broad inter-colony variation in mean colony growth rates including increased biomass, rates of calcification and branch growth (linear extension) for each host–symbiont combination (figure 2*h–j*). This variability is typical for most corals and is probably influenced by genotypic differences, phenotypic plasticity, microenvironmental conditions and some combination of these factors [11,52]. However, these inter-colony variabilities were remarkably similar for each mutualism, showing no differences in any growth measurements (figure 2*h–j*, electronic supplementary material, figure S1).

The only biological metric that indicated a physiological discrepancy between each mutualistic combination was the difference in mean egg sizes. While relative fecundity did not differ between colonies with either symbiont (figure 2*f*), at the July sampling, colonies with *D. glynnii* had slightly smaller oocytes than colonies harbouring *C. latusorum* (figure 2*g*). This observation is similar to previous findings where egg size and number appeared uncorrelated [17,52]. Because this incongruity may be explained by differences in the timing of oocyte maturation, additional temporal sampling is needed to assess whether this difference remained constant throughout oocyte maturation and spawning. Ultimately, tracking fertilization success, larval survivorship and settlement rate is required to determine whether egg number and size represent accurate proxies for colony health and reproductive fitness. In some studies, coral oocyte sizes correlated positively with energy reserves, such as lipids [53], and was assumed to be indicative of the animal's energetic health [17], but other studies found that larger, more developed, oocytes do not always correlate with higher lipid concentrations [54]. Differences in oocyte

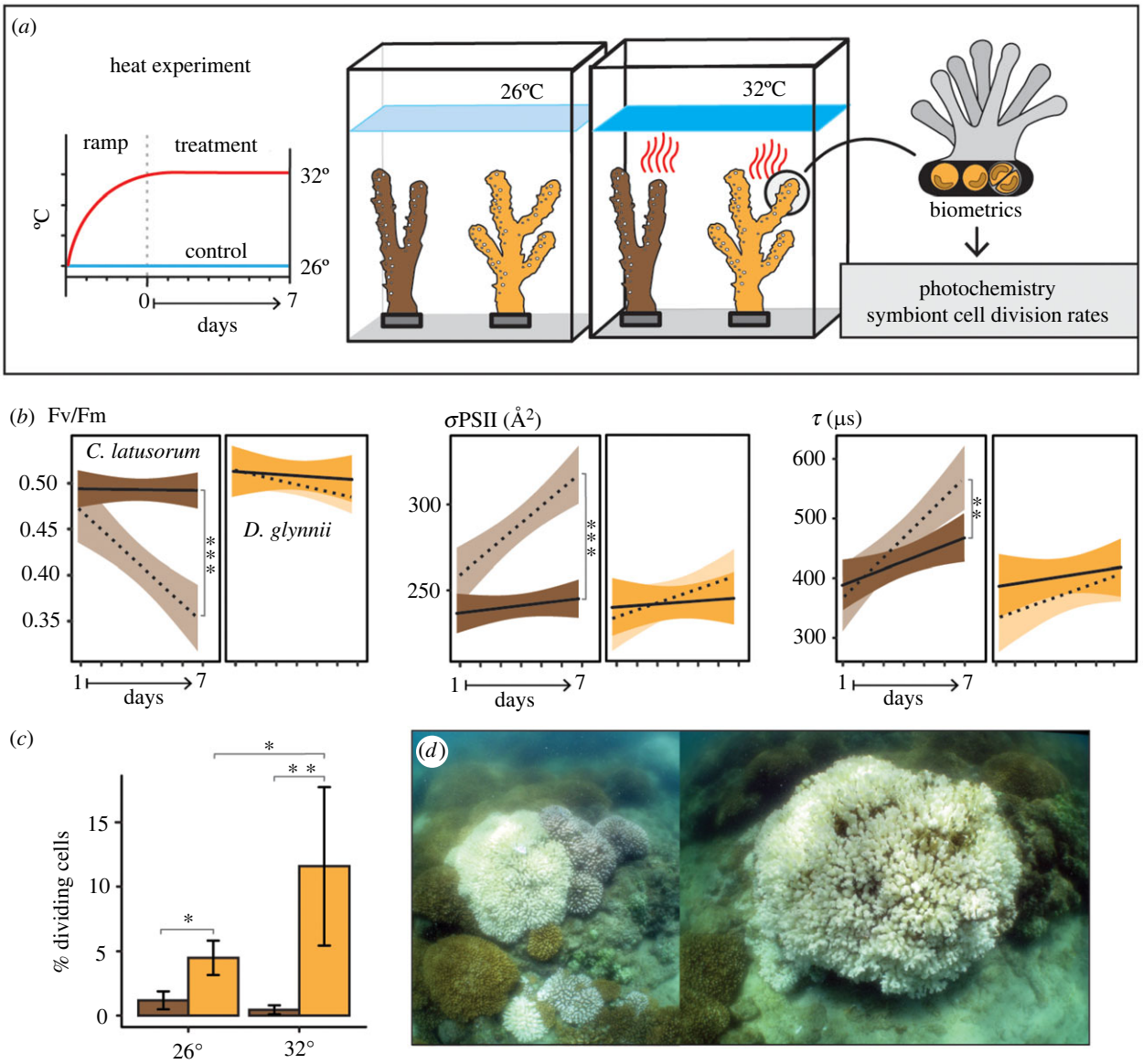

Figure 3. Differential response to thermal stress for each host–symbiont combination. (*a*) Experimental design for a thermal stress experiment. Replicate fragments from each of 6 colonies with *Durusdinium glynnii* and 6 colonies with *C. latusorum* were divided into treatment tanks incrementally ramped to 32°C, and control tanks which remained at 26°C. Colonies were then monitored for 7 days. (*b*) Colonies with *D. glynnii* exposed to 32°C exhibited no differences in photochemistry relative to controls ($p > 0.2$). By day 7, F_v/F_m decreased ($p < 0.001$), the functional absorption cross-section (σ) increased ($p < 0.001$), and the rate of reoxidation of the primary quinone acceptor $-Q_A$ of PSII (τ) was slower ($p = 0.002$) for colonies with *Cladocopium latusorum* relative to controls. Darker colours represent controls and lighter exposed to 32°C. (*c*) After 7 days at 32°C, peak mitotic indices for *D. glynnii* were significantly higher than for cells at control temperatures ($p = 0.038$), while the mean mitotic index for *C. latusorum* lowered significantly relative to controls ($p = 0.004$). All error bars and shaded regions represent one standard deviation. (*d*) Field images showing differential bleaching among *P. grandis* colonies with *C. latusorum* (bleached) versus those with *D. glynnii* (pigmented) after cold water anomaly in 2007 and 2008.

size may affect dispersal success to near and/or distant habitats [55]. Thus, size variation could be advantageous in some situations and increase the probability of survival in unpredictable environments [56].

(ii) Differences in seasonal acclimatization

Water temperatures and day length oscillate substantially between summer and winter in the Gulf of California [57]. Changes in these physical conditions appear to differentially affect cell division rates of *C. latusorum* and *D. glynnii*. With each species having different thermal and light optima for growth, seasonal differences in resource availability likely influence cell proliferation [58,59]. Additionally, differences between symbiont species in cell size and *in hospite* densities likely regulate their acquisition of inorganic nutrients, which may alter their physiological condition [60]. However, while seasonal cell division differed between these symbionts, Pettay *et al.* [61] and McGinley *et al.* [31,62] found their intra-colony dominance remained stable in tagged colonies during and after thermal stress events [61,62]. Most colonies are overwhelmingly dominated by one symbiont, with the other species often detected at trace levels (less than 1% of total symbiont cells in a colony; figure 1) [62]. Not only does one symbiont species persist [30,34,62], this stability often extends to the symbiont's genotype or clonal cell line. While rapid shifts in symbiont dominance sometimes occur in a subset of *Pocillopora* colonies (less than 5%), shifts were random, not favouring a particular symbiont over the other, and noted only after a stressful cold-water episode [62].

Table 2. Comparisons of seasonal mitotic indices (MI), per cent change in buoyant weights after six months and one year, as well as measures during the thermal experiment of MI, F_v/F_m, σ_{PSII} and τ comparing *Cladocopium latusorum* to *Durusdinium glynnii*. Two-way ANOVAs were used with fixed effects including species, date, and in the thermal experiment, treatment (experimental versus control), with fragment as a random effect. Variables had d.f = 1. Significant *p*-values ($p < 0.05$) are in bold. *Post-hoc* analyses were run for significant interactions. – denotes not applicable.

	% Δ in buoyant weight	seasonal MI	thermal expt. MI	thermal expt F_v/F_m	thermal expt σ_{PSII}	thermal expt τ
species	0.98	0.29	**0.005**	**<0.001**	**<0.001**	**0.001**
date	**<0.001**	0.21	0.21	**<0.001**	**<0.001**	**0.002**
treatment	–	–	–	**0.005**	**<0.001**	0.70
species × date	0.92	**<0.001**	–	0.093	0.24	0.22
species × treatment	–	–	0.11	**0.007**	**<0.001**	**0.021**
treatment × date	–	–	–	**0.038**	0.064	0.065
species × treatment × date	–	–	–	0.092	0.70	0.88

(b) Functionally similar mutualisms differed by physiological stressors

While colonies representing each mutualism had similar growth and fecundity, they differed significantly in their response to high temperature, which was driven by the different physiological tolerances of each symbiont to thermal stress. *D. glynnii* appeared largely unaffected by short-term exposure to 32°C (figure 3*b*). Moreover, large increases in cell division rates suggested that cellular processes were enhanced at this temperature (figure 3*c*). By contrast, *C. latusorum* experienced photodamage as evidenced by the significant decline in PSII efficiency (F_v/F_M), increased functional absorption cross-section, slower rate of electron transport (figure 3*b*) as well as a reduction in RNA transcripts for core photosystem reaction centre proteins [31]. These findings are consistent with the responses of each host–symbiont combination observed during natural episodes of thermal stress (figure 3*d*) where *D. glynnii* cell densities remained unchanged in colonies exposed to thermal stress, whereas affected colonies with *C. latusorum* experienced significant cell losses [9,63]. Indeed, in places across the Pacific Ocean, pocilloporid and montiporid colonies with *D. glynnii* tend to bleach less and experience lower mortality than colonies hosting *Cladocopium* spp. [1,9,10,30]. Differential mortality over the course of numerous thermal anomalies in the Eastern Pacific since the early 1980s may largely explain why there are far fewer colonies with *C. latusorum* in the region compared to those with *D. glynnii* [1,9,64].

It is long established that *Durusdinium* mutualisms are better at tolerating stressful environmental conditions (i.e. marine heat waves). Colonies harbouring them often thrive under environmental conditions deemed suboptimal for many reef corals [6,25,61,65–67]. Continued mutualistic functioning under high temperatures, for example, imparts greater stability to the mutualism as a whole. Despite this, there are few known attributes, genetic or physiological, that explain the stress tolerance of these symbionts. Recent evidence attributes *Durusdinium* thermal tolerance to increased nitrogen assimilation at higher temperatures [14], as well as saturation in specific lipids known for stabilizing PSII structure (e.g. sulfoquinovosyldiacylglycerols, SQDGs) and stabilizing thylakoid membranes by increasing the monogalactosyl diacylglycerol and digalactosyl diacylglycerol ratio (DGDG: MGDG) [68]. Smaller *D. glynnii* cell volume probably enhances nutrient acquisition and therefore cell growth, which may contribute to its thermal tolerance and population maintenance *in hospite* [69,70], but a complete understanding of the underlying cellular and biochemical mechanisms responsible for thermal tolerance in *D. glynnii* remains incomplete.

(c) Not all corals with thermally tolerant symbionts have significant physiological tradeoffs

Our results as well as those from other studies indicate that colonies hosting *D. glynnii* do not exhibit significant metabolic tradeoffs in exchange for increased thermal tolerance (figure 2) [19,20]. Colonies with *D. glynnii* in the Eastern Pacific are highly abundant with sizes reaching 1–3 m across. Compared to *Pocillopora* colonies typical in other regions of the central and west Pacific, the robust growth and unusual colony size indicate that this mutualism is well adapted to the region's environment (figure 1; personal observation). The adaptive radiation of *Durusdinium* in the Indo-West Pacific during the Pleistocene led to the evolution of numerous ecologically specialized species like *D. glynnii* that maintain stable associations with specific host taxa [11,25,65]. Moreover, facilitated by vertical transmission, pocilloporid corals share a close evolutionary history with their symbionts, which further explains their high-functioning mutualism with *D. glynnii* [26,71]. These evolutionary factors reconcile the present findings with previous reports of significant growth trade-offs among corals with thermally tolerant symbionts [15–17].

Previous reports of growth tradeoffs are context dependent and possibly explained by different factors, including the geographical location and species identity of the *Durusdinium* under study. Juvenile *Acropora* colonies experienced almost 50% reduced growth (measured as the number of new polyps added over time) when associated with *Durusdinium* sp. on the Great Barrier Reef [16]. Moreover, adult colonies had 25–30% fewer lipid stores and smaller oocytes than colonies harbouring *Cladocopium* spp. [17]. Lower rates of translocated photosynthate accounts for the reduced growth,

low energy reserves and smaller oocyte sizes measured in these GBR *Acropora* colonies [15]. The *Acropora-Durusdinium* combinations found on the GBR are rare, however, and occur in the most marginal of reef habitats [15–17,72,73], indicating that the environments of this reef system do not readily support mutualisms involving this genus of symbiont. Similarly, Caribbean *Orbicella* colonies hosting *Durusdinium trenchii* exhibit approximately 50% lower calcification rates ([18], but see also [74]), have lower tissue biomass, and fewer nutrient reserves (D. W. Kemp 2023, personal communication). However, *D. trenchii* is a non-native species recently introduced from the Indo-Pacific [18]. Ultimately, earlier characterizations of poor growth seem to relate to rare or introduced species of *Durusdinium*, representing maladapted mutualisms lacking a coevolutionary history.

Arguably, physiological performance is expected to be optimized in host corals like *Pocillopora* which have coevolved for hundreds of thousands to millions of years with symbionts in the genus *Durusdinium* [25]. As research expands to more tropical warm-water regions of the Indo-Pacific, these host–symbiont pairings do not appear to experience reduced translocation or calcification (D. W. Kemp *et al.* 2023, unpublished data). *D. glynnii* in *Pocillopora* supports high rates of photosynthesis per cell and maintains carbon translocation under higher temperature [23]. Indeed, a clearer understanding of the natural history of reef corals and their symbionts, including the discernment of closely related symbiont species, is necessary to better explain physiological and ecological patterns and processes.

(d) Host dependency on co-evolved symbionts in a changing climate

These findings provide insight into the persistence of reef corals as oceans continue to warm and marine heat waves increase in frequency [75,76]. Because certain symbiont species raise the thermal tolerance of host corals by 1–2°C, different host–symbiont partner combinations induce phenotypic change faster than evolutionary adaptation. This ecological response is limited by partner specificity and symbiont availability [6,77–79], however, many stress-tolerant symbionts, including *D. glynnii*, are widespread throughout the Indo-Pacific [65,66]. In some mutualisms, harbouring symbionts with physiological adaptations that allow tollerance to high temperatures can result in metabolic tradeoffs affecting colony growth and fitness. By contrast, the physiological integration between co-evolved mutualisms, like *Pocillopora* and *D. glynnii*, creates fast-growing, highly fecund, colonies with few observable trade-offs. The larger implications of these findings are numerous. Increased prevalence of these mutualisms under higher or more variable ocean temperatures may continue to support ecosystem productivity and contribute to reef growth [33]. Future comprehensive analyses, such as the present case study, will offer better insights into the functional ecology of coral–dinoflagellate mutualisms and how partner diversity in these relationships creates important variation critical in their responses to climate change.

Data accessibility. All data are provided in the online electronic supplementary material, which is deposited in Dryad: https://doi.org/10.5061/dryad.b8gtht7j0 [80]. All R code used in analyses are available via github at the following link: https://github.com/Kira-Turnham/Pocillopora-thermal-tolerance.

Additional information is provided in electronic supplementary material [81].

Authors' contributions. K.E.T.: data curation, formal analysis, investigation, methodology, project administration, resources, software, validation, visualization, writing—original draft, writing—review and editing; M.D.A.: data curation, investigation, methodology, validation, writing—review and editing; D.T.P.: data curation, investigation, methodology, validation, writing—review and editing; D.A.P.-G.: investigation, methodology, validation, writing—review and editing; H.R.-B.: investigation, methodology, resources; J.P.: data curation, funding acquisition, investigation, methodology, validation, writing—review and editing; E.T.: data curation, validation; R.T.S.: data curation, investigation, methodology, validation, writing—review and editing; M.P.M.: data curation, investigation, methodology, validation, writing—review and editing; M.E.W.: conceptualization, data curation, funding acquisition, investigation, methodology, project administration, resources, supervision, validation, writing—review and editing; T.C.L.: conceptualization, data curation, funding acquisition, investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing—original draft, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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