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REPORT

Plasticity of symbiont acquisition in new recruits of the massive coral *Platygyra daedalea* under ocean warming and acidification

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Abstract Symbiosis establishment is a milestone in the life cycles of most broadcast-spawning corals; however, it remains largely unknown how initial symbiont infection is affected by ocean warming and acidification, particularly for massive corals. This study investigated the combined effects of elevated temperature (29 vs. 31 °C) and pCO_2 ($\sim$ 450 vs. $\sim$ 1000 µatm) on the recruits of a widespread massive coral, *Platygyra daedalea*. Results showed that geometric diameter and symbiosis establishment were unaffected by high pCO_2, while elevated temperature significantly reduced successful symbiont infection by 50% and retarded the geometric diameter by 6%. Although neither increased temperature, pCO_2, nor their interaction affected survival or algal pigmentation of recruits, there was an inverse relationship between symbiont infection rates and survivorship, especially at high temperatures, possibly as a result of oxidative stress caused by algal symbionts under increased temperature. Intriguingly, the proportion of *Durusdinium* did not increase in recruits at 31 °C, while recruits reared under high pCO_2 hosted less *Breviolum* and more *Durusdinium*, indicating a high degree of plasticity of early symbiosis and contrasting to the previous finding that heat stress usually leads to the prevalence of thermally tolerant *Durusdinium* in coral recruits. These results suggest that ocean warming is likely to be more deleterious for the early success of *P. daedalea* than ocean acidification and provide insights into our understanding of coral-algal symbiotic partnerships under future climatic conditions.

Keywords Symbiosis plasticity · *Platygyra daedalea* · Symbiodiniaceae · Ocean warming · Ocean acidification

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Introduction

The obligate association between reef corals and unicellular algal symbionts from the family Symbiodiniaceae lays the nutritional and structural foundations for coral reef ecosystems. However, corals, and the reef ecosystems they construct, are currently confronted with two major challenges, ocean warming and acidification (OW & OA) driven by the ever-increasing atmospheric carbon dioxide (CO_2) concentrations (Hoegh-Guldberg et al., 2007). Warming-induced mass coral bleaching events and subsequent coral mortality have been extensively documented over a large geographic scale, contributing greatly to the

global degradation of coral reefs (Hoegh-Guldberg 1999; Bellwood et al., 2004; Hughes et al., 2017). OA is another threat to the persistence of scleractinian corals and the reefs they build, impairing productivity, calcification and recruitment, and causing reef dissolution (Anthony et al., 2008; Andersson et al., 2009; Albright et al., 2010; Albright and Langdon 2011).

At the current rates of carbon emissions, seawater temperatures are projected to increase by 2− 3 °C by the end of this century, and pCO_2 is expected to double, thus putting corals and coral reefs at unprecedented risk (Hoegh-Guldberg et al., 2007, 2017). Reef-building corals are sessile invertebrates, with a sequential recruitment process that involves fertilization, larval development, dispersal and settlement onto benthic substrates, and the post-settlement growth into juveniles (Harrison 2011). Sufficient levels of recruitment, particularly the successful post-settlement growth and survival, are critical to the maintenance and replenishment of coral populations, as well as the recovery of damaged reefs following disturbances (Ritson-Williams et al., 2009). It is, therefore, vitally important to understand how these early stages of reef corals will be affected by the near-future increases in temperature and/or pCO_2.

Elevated temperature and pCO_2 will interact concurrently on reefs in the future, yet there are comparatively few studies examining the combined effects of these two stressors on the early stages of corals. For instance, coral fertilization success will be greatly compromised under combinations of increased temperature and pCO_2, especially when sperm availability is limited (Albright and Mason 2013). Others have shown that increasing temperatures and high pCO_2 can affect larval survival, metabolism, and settlement (Chua et al., 2013; Putnam et al., 2013; Rivest and Hofmann 2013, 2015; Jiang et al., 2020; Pitts et al., 2020). Moreover, increased temperatures either partially mitigate or synergistically amplify the negative effects of seawater acidification on post-settlement growth and calcification (Anlauf et al., 2011; Foster et al., 2015, 2016; Jiang et al., 2018).

Over 80% of all the broadcast-spawning corals produce azooxanthellate eggs and planula larvae, which must capture symbionts (Symbiodiniaceae) from the surrounding environment to form obligate symbiosis, a pattern which is termed horizontal transmission (Baird et al., 2009). In such a way, corals could acquire genetically varied symbionts, including those with different environmental tolerances which will potentially enable the holobiont to acclimatize to local conditions, thus enhancing the fitness of symbiosis (Douglas 1998). To date, nine genera in the family Symbiodiniaceae, previously designated as Clades A–I, have been formally defined based on a suite of genetic markers and ecological and morphological traits (LaJeunesse et al., 2018; Nitschke et al., 2020). In general, algal symbionts from *Symbiodinium* (Clade A), *Breviolum* (Clade B), *Cladocopium* (Clade C), *Durusdinium* (Clade D) and *Fugacium* (Clade F) have been found in symbiosis with reef corals, with each genus containing several phylotypes (LaJeunesse et al., 2018).

Numerous field and laboratory studies have found that larval and juvenile corals exhibit a dynamic association with zooxanthellae, and they can harbor a broader range of symbiont types than those found in the corresponding adult phase (Coffroth et al., 2001; Goulet and Coffroth 2003; Little et al., 2004; Gómez-Cabrera et al., 2008; Abrego et al., 2009b,a; Cumbo et al., 2013; McIlroy and Coffroth 2017; Poland and Coffroth 2017; Ali et al., 2019). In contrast, others demonstrated that some cnidarians exhibit a certain degree of specificity and selectivity for algal symbionts during early ontogeny (Weis et al., 2001; Mauricio et al., 2004; Hambleton et al., 2014; Yamashita et al., 2014). Furthermore, early establishment of symbiosis could dramatically influence the growth and survival of juvenile corals (Little et al., 2004; Suzuki et al., 2013; Yuyama and Higuchi 2014) and critically determine the performance of coral holobiont in the face of environmental changes (Abrego et al., 2008, 2012; Mieog et al., 2009; Howells et al., 2012; Yuyama et al., 2016).

While increasing information is available about the interactive effects of temperature and pCO_2 on the early stages of corals and the role of symbiont identity in their early responses to environmental stress, major knowledge gaps remain regarding the impact of warmer and less basic seawater on symbiont acquisition and selection. Only a handful of studies have investigated the impact of elevated temperature and/or pCO_2 on symbiosis establishment in early stages of corals (Baird et al., 2010; Suwa et al., 2010; Abrego et al., 2012; Schnitzler et al., 2012; Yorifuji et al., 2017; Cumbo et al., 2018; Sun et al., 2020). A general emerging pattern is that symbiont infection of coral larvae and recruits is susceptible to elevated temperature, and that sensitivity of symbiont uptake success to thermal stress is heavily dependent upon host species, symbiont genotypes, and other abiotic factors, such as light and temperature (Baird et al., 2010; Abrego et al., 2012; Schnitzler et al., 2012; Yorifuji et al., 2017; Cumbo et al., 2018; Sun et al., 2020). Furthermore, elevated temperatures generally favor the uptake of thermotolerant *Durusdinium* (formerly clade D) and alter the symbiont communities within juvenile corals (Abrego et al., 2012; Yorifuji et al., 2017; Sun et al., 2020). On the other hand, the effects of OA on symbiont infection have so far been equivocal, with evidence showing that elevated pCO_2 delays symbiont acquisition in primary polyps of *Acropora digitifera* (Suwa et al., 2010), while such an effect was not observed in newly settled *A. intermedia* (Sun et al., 2020). Despite this recent progress,

there is still very limited understanding about the combined impact of increased temperature and pCO_2 on the symbiont uptake process and particularly the plasticity of early symbiosis. In addition, studies exploring the effects of temperature and pCO_2 on coral recruits mainly focus on species from the complex clade with fast growing rates and porous skeletal structures (e.g., *Acropora* and *Porites*), and few have examined the responses of early stages of robust spawning corals to ocean warming and/or acidification (Williamson et al., 2021).

Most robust corals are broadcast spawners (Harrison 2011), and more importantly, they are often rated as "winners" in bleaching events because of a better capacity to fare well through thermal anomaly (Loya et al., 2001; Depczynski et al., 2013). Scrutinizing how juvenile corals of the robust clade will be affected by the projected temperature and carbonate chemistry has important implications for our understanding of the breadth of response variability and the possible shift in coral community structure in the near-future.

The objective of this study was to investigate the combined effects of temperature and pCO_2 on the early stages of a massive coral *Platygyra daedalea*. Newly settled primary polyps of *P. daedalea* were incubated in flow-through seawater tanks where temperature and pCO_2 were controlled. We assessed the growth, survival, and symbiont infection of recruits and further examined the plasticity and flexibility of early coral-Symbiodiniaceae symbiosis in new recruits of *P. daedalea* under thermal and pCO_2 perturbations.

Materials and methods

Coral sampling and preparation of new recruits

Four gravid colonies of *P. daedalea* were collected from Luhuitou fringing reef (18°12′N, 109°28′E) and they spawned at 21:00 on May 30, 2016. Egg-sperm bundles were gently mixed for cross-fertilization. Larvae were raised in 0.5-µm filtered seawater. Eight-day-old larvae were introduced to 14-cm-diameter petri dishes and small chips of crustose coralline algae *Hydrolithon onkodes* were added to induce settlement. Two days later, two to three dishes with a total of 37 − 46 recruits (16 ± per dish, mean ± SE, $n = 21$) were randomly assigned to each experimental tank.

Experimental setup

Treatments consisted of two temperatures and two pCO_2 levels in a factorial crossed design as follows: (1) 29 °C, ambient CO_2 (ATAC), (2) 29 °C, high CO_2 (ATHC), (3) 31 °C, ambient CO_2 (HTAC), (4) 31 °C, high CO_2 (HTHC). The 29 °C was the ambient temperature during the spawning of *P. daedalea* and approximates the average summer seawater temperature at the study site. The ambient CO_2 treatment (450 µatm) was representative of the present-day pCO_2 on Luhuitou fringing reef (Zhang et al., 2013). The future scenario treatments achieved a temperature increase of 2 °C and pH decline of 0.3 units, which were close to the projections for the northern South China Sea by 2100 (Bopp et al., 2013; Gattuso et al., 2015). There are great seasonal temperature fluctuations (19.9–33.4 °C) on Luhuitou reef (Li et al., 2012), and while our experimental temperature of 31 °C was below the summer maximum, it resulted in a cumulative heat stress of 8 degree heating weeks (DHW).

Seawater was pumped from approximately 3 m depth on the reef where adult corals were collected, and then it was passed through a sand-filter before entering the experimental tanks. Treatments were created in duplicate 75 L tanks receiving sand-filtered seawater at a flow rate of 0.7 L min^{-1}. Once recruits were introduced into each tank, seawater temperature was elevated by 1 °C per day while pH was reduced by 0.15 unit per day, after which seawater temperature and pH were maintained. Water temperature was maintained using temperature controllers and titanium heaters (Weipro, China). The carbonate chemistry within high pCO_2 tanks was manipulated with pH regulators (Weipro, China) via a solenoid valve to control the bubbling of CO_2 from compressed CO_2 tanks. High-resolution (every 15 min for 8 h) pH monitoring revealed that the precision of pH control was less than 0.05, and pH did not vary between replicate tanks within treatments ($F_{4,\ 256} = 0.26$, $P = 0.90$). Submerged pumps (600 L h^{-1}) were used to ensure good circulation and mixing of seawater within tanks. Each tank was illuminated from 07:00 to 19:00 with a series of full spectrum, T5 fluorescent bulbs (Giesemann). Light intensity at the tank bottom averaged 200 ± 3 µmol photons m^{-2} s^{-1}, which is close to that in crevices where coral recruits preferred at 3 m depth in Luhuitou fringing reef (Lei Jiang, unpublished data).

Temperatures within each tank were continuously recorded using Hobo Pendant loggers at 15 min intervals. Salinity and pH were monitored three times a day using conductivity and pH meters (Mettler-Toledo Seven Go). Seawater samples (100 ml) were taken every 4 days from each tank and preserved with mercuric chloride. Total alkalinity was measured with a Gran titrator (Apollo SciTech Model AS-ALK2). Aragonite saturation state (Ω_{Arag}) and pCO_2 were calculated from the measured temperature, salinity, pH and alkalinity using CO2SYS (Lewis et al., 1998), with dissociation constants for carbonate determined by Dickson and Millero (1987) and the Dickson

constant for the HSO − 4 (Dickson 1990). The physical and chemical parameters are presented in Table 1.

Timing of symbiosis, survival, growth, and algal pigmentation

During the experiment, coral recruits were seeded with naturally occurring free-living symbionts in the flowing water column. Every 3 − 5 days, recruits were inspected under a dissecting microscope (Olympus, Japan) to characterize their symbiotic status. At each observation, polyps were scored for the acquisition of symbionts following Nitschke et al., (2016), and here we defined the presence of brown symbiont cells and visual pigmentation as infection and uptake success (Fig. 1). It is important to note that though this visual approach represents a good proxy for symbiosis establishment which incorporates both successful symbiont infection and proliferation within coral host (Abrego et al., 2012; Nitschke et al., 2016), it may not reflect the actual symbiont infection status in juvenile corals when symbiont abundance is extremely low (Sun et al., 2020). Meanwhile, survival was assessed based on the presence of polyp tissue. At the last day of the experiment, the maximum diameter and perpendicular diameter were measured for each recruit using the cellSens software under the dissecting microscope. Growth of recruits was assessed as the geometric mean of perpendicular diameters using the following equation: Geometric diameter $= \sqrt{\text{the maximum diameter} \times \text{perpendicular diameter}}$ (Kwok et al., 2016).

The extent of algal pigmentation was assessed photographically following Siebeck et al., (2006). At the end of the experiment, all symbiotic recruits were photographed under the Olympus dissecting microscope and identical illumination using the cellSens software. The saturation of each coral picture, a good proxy for symbiont density and algal pigmentation, was measured by taking the average saturation value on each coral picture using Photoshop's histogram function (Siebeck et al., 2006). The algal pigmentation extent was quantified as the percentage of saturation value of each recruit relative to the one yielding the maximum value.

Symbiont genotyping

At the end of the 28-d experiment, all pigmented juveniles from each treatment were scraped and rinsed three times in 0.5-μm filtered seawater and then preserved in 70% ethanol for DNA extraction. To compare the difference in Symbiodiniaceae composition between parent and offspring, a small fragment from each parent colonies (PC) was also sampled and preserved in 70% ethanol. Total DNA was extracted using the Fast DNA® SPIN Kit for Soil (MP Biomedicals, CA) according to the manufacturer's instructions. Purified DNA samples were kept at 20 °C until use.

The internal transcribed spacer 2 (ITS2) region of Symbiodiniaceae was amplified using a barcoded Symbiodiniaceae-specific primer: ITS2intfor (5′-GAATTGCAGAACTCCGTG-3′) and ITS2-reverse (5′-GGATCCATATGCTTAAGTTCAGCGGGT-3′) (Lajeunesse and Trench 2000). PCR amplification was performed with a thermocycle controller (MJ Research Inc., Bio-Rad) using the following program: 94 °C for 5 min; 35 cycles of 94 °C for 30 s, 51 °C for 30 s, and 72 °C for 30 s; and 72 °C for 5 min. All PCR products were further purified by DNA Fragment Purification Kit (Takara, Japan) and quantified with the NanoDrop spectrophotometer. All amplicon products were mixed in equal concentrations followed by sequencing on an Illumina Hiseq platform using 2 × 300 bp mode at Novogene (Beijing, China). The raw data were submitted to the NCBI Sequence Read Archive under accession numbers SRR14089794.

After demultiplexing in QIIME (Caporaso et al., 2010), paired reads from each sample were remotely analyzed with the SymPortal framework (SymPortal.org; Hume et al., (2019)), which discriminates between intragenomic and intergenomic variation using minimum entropy decomposition (MED) algorithm. The prediction of Symbiodiniaceae ITS2 type profiles (putative Symbiodiniaceae genotypes) was based on the presence and abundance of the ITS2 sequences within the SymPortal database. For more details of this method, refer to Hume et al., (2019). The counts of Symbiodiniaceae ITS2 type profiles output from the SymPortal framework were used for downstream statistical analysis. Datasets associated with

Table 1 Mean (± SD) physical and chemical conditions during the 4-week experiment

Treatment	Temperature X (°C)	pH_T	TA (μmol kg^{-1})	DIC (μmol kg^{-1})	pCO_2 (μatm)	Ω_{Arag}
ATAC	29.0 ± 0.3	7.99 ± 0.04	2300 ± 14	2016 ± 24	479 ± 50	3.38 ± 0.24
ATHC	29.1 ± 0.3	7.69 ± 0.05	2284 ± 25	2138 ± 20	1012 ± 80	1.97 ± 0.15
HTAC	30.8 ± 0.4	7.98 ± 0.04	2297 ± 18	1984 ± 25	457 ± 38	3.72 ± 0.19
HTHC	30.8 ± 0.3	7.69 ± 0.05	2273 ± 28	2122 ± 25	1044 ± 114	2.04 ± 0.18

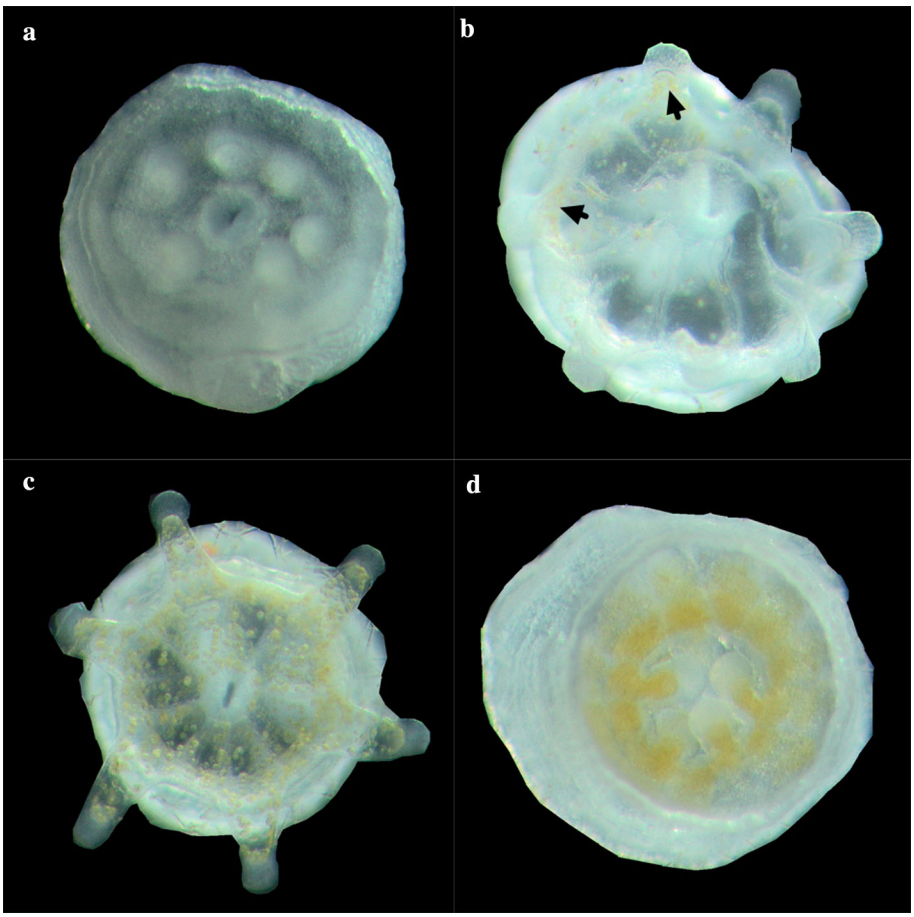

Fig. 1 **a** Two-day-old aposymbiotic polyp, **b** 4-day-old polyp with Symbiodiniaceae (arrows) visible within oral disk and tentacles, **c** 8-day-old, and **d** 4-week-old symbiotic polys of *P. daedalea*

Symbiodiniaceae ITS2 type profiles are available in the supplementary material.

Statistical analysis

Since temperature and pCO_2 in each tank were manipulated independently, we first included tank as a random effect nested within temperature and pCO_2. When tank effects were non-significant, they were dropped from the statistical model to enhance power of the analysis (Underwood 1997). To test for the impact of temperature and pCO_2 on symbiont infection success and survival, we used three-way ANOVAs with repeated measures. Temperature and pCO_2 were fixed factors, and time was the within subject factor. When there was a significant interaction between time and temperature or pCO_2, separate analyses were performed at each time point. Differences in algal pigmentation and geometric diameter among treatments were compared using a nested two-way ANOVA. Each petri dish served as a replicate in the analyses of symbiont infection success and survival, while each recruit was treated as a replicate in ANOVAs on algal pigmentation and geometric diameter. When the main effects were significant ($P < 0.05$), planned multiple comparisons were conducted using Fisher's least significant difference (LSD) tests following ANOVAs. Permutational analysis of variance (PERMANOVA) was used to test for differences in Symbiodiniaceae community between treatments. Principal component analysis of Bray–Curtis dissimilarities of counts was used to illustrate the variation in Symbiodiniaceae compositions among treatments.

Results

Symbiont uptake success

Visibly infected polyps, with brown symbiont cells present in tentacles, were first observed after 4 d at ambient temperature (Fig. 1). Eight days after incubation, about 40% of polyps at 29 °C, regardless of pCO_2, had acquired symbionts, while there were only 10% and 25% symbiotic polyps in HTAC and HTHC, respectively. Four weeks later, the proportions of pigmented recruits were 88% and 83%, respectively, in ATAC and ATHC. In comparison, these percentages were reduced by almost 50% at 31 °C (Fig. 2a). Elevated temperature significantly affected the symbiont infection and uptake success, however, neither

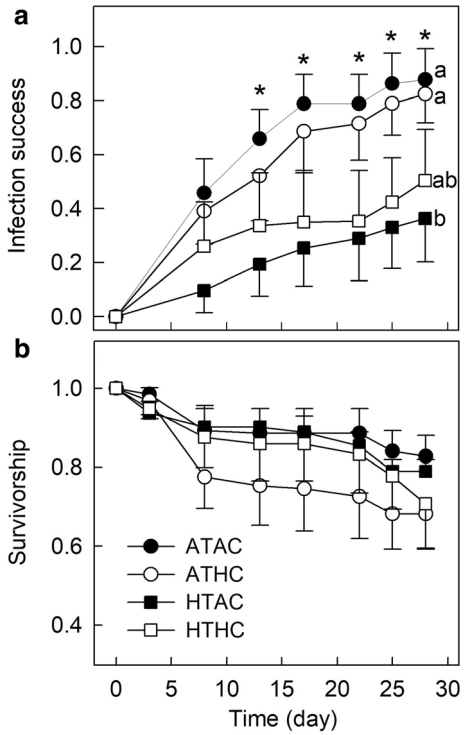

Fig. 2 **a** Successful symbiont acquisition, **b** survivorship for *P. daedalea* recruits under combinations of temperature (29 °C, 31 °C) and pCO_2 ($\sim$ 450 µatm, $\sim$ 1000 µatm). Error bars are SE (n = 5 − 6 per treatment, with each petri dish as a replicate). *Asterisks* indicate significant effects of temperature on infection success at a specific time determined by separate two-way ANOVAs. Different letters indicate significant differences as determined by Fisher's LSD post hoc comparisons

pCO_2 nor the interaction between temperature and pCO_2 influenced symbiosis establishment (Table 2).

Post-settlement survival and growth, and algal pigmentation

After 4 weeks of exposure, percent survival ranged from 68% in ATHC to 83% in ATAC (Fig. 2b). There was no significant effect of temperature, pCO_2 or their interaction on survivorship (Table 2). When survival was plotted against successful symbiont infection rates, there was a trend toward lower survivorship with increasing infection rates (Fig. 3a), especially at 31 °C (Fig. 3b). However, no statistically significant relationship was found between survival and infection success at 29 °C (Fig. 3c).

The extent of pigmentation which serves as a proxy for symbiont density was similar among treatments and was unaffected by temperature, pCO_2 and their interaction (Fig. 4a, Table 2). Elevated pCO_2 alone had no effect on the lateral growth of recruits, while geometric diameter significantly declined at increased temperature (Table 2); however, the effect size was small, only amounting to a 6% reduction at 31 °C (Fig. 4b). At 29 °C, high pCO_2 did not impact diameter, while it tended to mitigate the negative effect of temperature on growth at 31 °C (Fig. 4c).

Symbiodiniaceae compositions within juvenile and adult corals

A total of 17 Symbiodiniaceae ITS2 type profiles were identified in both juvenile and adult *P. daedalea*. Symbiodiniaceae community compositions in parent and juvenile were significantly different (PERMANOVA, $P < 0.01$). Adult *P. daedalea* associated predominantly with a single *Cladocopium* ITS2 type profile C50c/C50a/C3-C50f-C3b-C50u-C3ad, representing nearly 90% of the sequences, whereas juveniles contained multiple Symbiodiniaceae ITS2 type profiles belonging to different genera, which were mainly compromised of *Symbiodinium*, *Breviolum,* and *Durusdinium* (Fig. 5, electronic supplementary material, ESM). The *Cladocopium* ITS2 type profiles in juvenile corals only had an abundance lower than 0.7%. Furthermore, Symbiodiniaceae compositions within juveniles also differed among temperature and pCO_2 treatments. Specifically, the decrease in the relative abundance of *Symbiodinium* ITS2 type profile A3-A6b-A3g-A6i was accompanied by a disproportionate increase in the relative abundance of *Breviolum* and *Durusdinium* ITS2 type profiles at elevated temperature or pCO_2. Furthermore, high temperature increased the relative abundance of *Breviolum* at ambient pCO_2, while high temperature decreased the relative abundance of *Breviolum* at high pCO_2 (ESM). Notably, the relative abundance of *Durusdinium* ITS2 type profile D1-D4-D4c-D1h was dominant in juvenile corals exposed to elevated pCO_2 at both temperatures (ESM).

Discussion

Most reef corals produce offspring which initially lack symbionts, and thus they need to capture symbionts each new generation, a strategy which allows for the acquisition of symbionts that are optimal for the environmental conditions in which they settle (Douglas 1998; Baird et al., 2009; Cumbo et al., 2018). Here, we showed that increased temperature delayed uptake success of symbionts in newly settled *P. daedalea,* while high pCO_2 had no detectable effect. Neither increased temperature, pCO_2, nor the combination of both affected survival of recruits. While geometric diameter was unaffected by pCO_2, it was reduced at increased temperature, possibly as a result of delayed symbiosis establishment under higher temperature. Furthermore, elevated temperature and pCO_2 altered the compositions of symbiont community within recruits,

Table 2 Analysis of variance of symbiont infection rates, survival, and growth of *Platygyra daedalea* recruits under the combinations of two levels of temperature (~ 29 °C and ~ 31 °C) and pCO_2 (~ 450 µatm and ~ 1000 µatm)

Source of variation	SS	*df*	MS	*F*	*P*
Infection success					
Within subject					
Time	1.629	5	0.322	23.51	**< 0.001**
Time × Temp	0.171	5	0.034	2.50	**0.037**
Time × pCO_2	0.016	5	0.003	0.230	0.947
Time × Temp × pCO_2	0.016	5	0.003	0.230	0.947
Between subject					
Temp	4.629	1	4.629	8.16	**0.011**
pCO_2	0.08	1	0.008	0.010	0.906
Temp × pCO_2	0.18	1	0.318	0.560	0.464
Survivorship					
Within subject					
Time	0.57	6	0.09	19.5	**< 0.001**
Time × Temp	0.04	6	0.01	1.40	0.22
Time × pCO_2	0.04	6	0.01	1.31	0.26
Time × Temp × pCO_2	0.01	6	0.00	0.46	0.83
Between subject					
Temp	0.03	1	0.03	0.24	0.63
pCO_2	0.21	1	0.21	1.88	0.19
Temp × pCO_2	0.05	1	0.08	0.72	0.41
Algal pigmentation					
Temp	0.114	1	0.114	3.648	0.058
pCO_2	0.010	1	0.010	0.320	0.572
Temp × pCO_2	0.00001	1	0.00001	0.001	0.981
Geometric diameter					
Temp	47,083	1	47,083	4.41	**0.037**
pCO_2	5513	1	5513	0.52	0.473
Tank (Temp × pCO_2)	82,506	4	20,627	1.93	0.106
Temp × pCO_2	4958	1	4958	0.46	0.496

Significant results are highlighted in bold

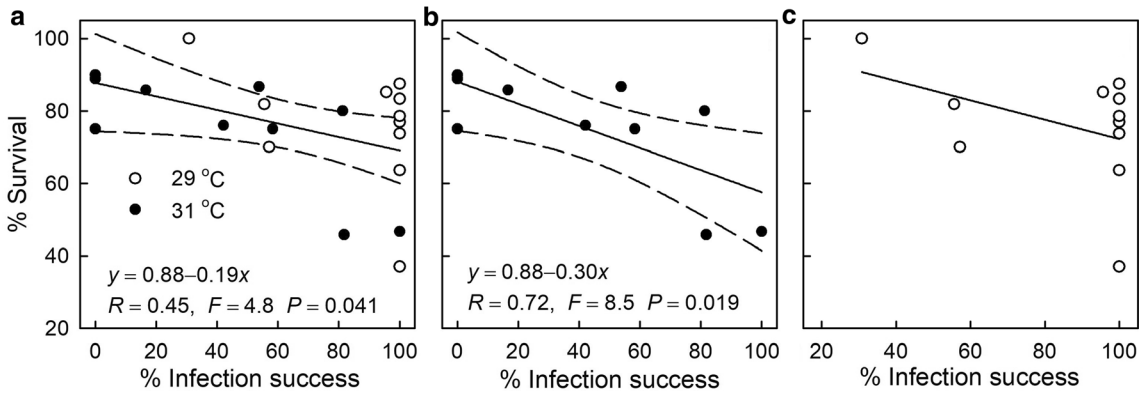

Fig. 3 Relationships between percent infection success and survival of *P. daedalea* recruits at **a** both temperatures, **b** 31 °C, and **c** 29 °C. Dashed lines indicate 95% confidential intervals

suggesting a certain degree of symbiosis plasticity to thermal and pCO_2 perturbations.

Unlike most prior studies that directly inoculated larval and juvenile corals with cultured or freshly isolated symbionts, the present study employed a flow-through system to examine how corals select free-living symbionts from natural seawater to establish symbiosis under increased temperature and pCO_2. The proportion of *P.*

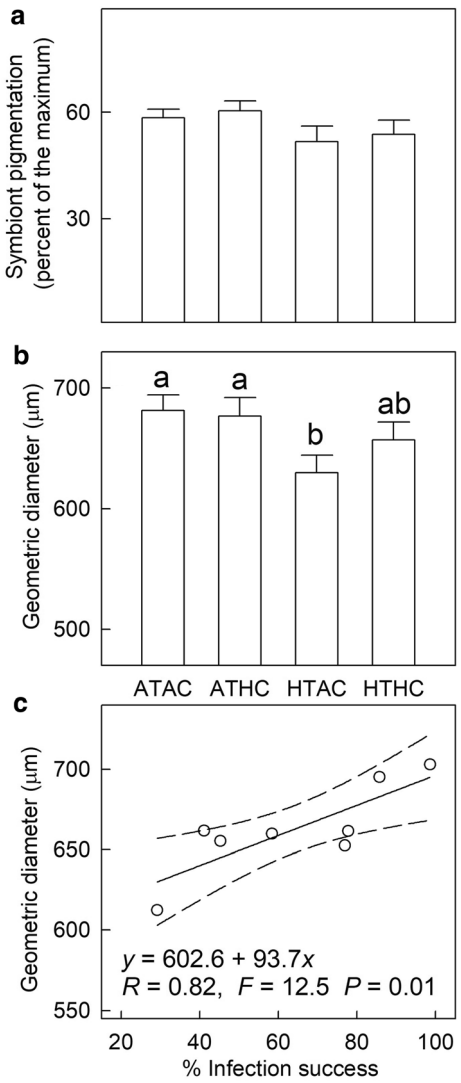

Fig. 4 **a** Algal pigmentation and **b** geometric diameter of 28-day-old *P. daedalea* recruits under combinations of temperature (29 °C, 31 °C) and pCO_2 (~ 450 µatm, ~ 1000 µatm); **c** the relationship between geometric diameter and percent infection rates. Error bars are SE (n = 46–66 per treatment, with each recruit as a replicate). Data points in **c** represent mean values for each replicate tank and dashed lines indicate 95% confidential intervals. Different letters indicate significant differences as determined by Fisher's LSD post hoc comparisons

daedalea recruits with visible symbiont pigmentation reached 87.7% after 4 weeks in flow-through tanks at ambient conditions. This ratio is commensurate with those reported for juvenile *A. tenuis* and gorgonian corals deployed in the field (Coffroth et al., 2001; Graham et al., 2013). Although numerous lines of evidence have shown that reef sediment constitutes an important Symbiodiniaceae pool for primary symbiont acquisition in corals (Littman et al., 2008; Adams et al., 2009), symbiont cells should first migrate into seawater before uptake and infection in newly settled corals (Nitschke et al., 2016). Therefore, the natural seawater flow-through experimental system used here seems adequate to study symbiont uptake process in coral recruits. However, the rate of symbiont acquisition was much faster for *P. daedalea* in this study than other reports. Thirteen days later, about 66% of *P. daedalea* recruits successfully established symbiosis at ATAC. In contrast, only between 10 and 20% of *Acropora* spp., *Isopora palifera*, *Orbicella faveolata,* and *Pseudodiploria strigosa* had acquired symbionts after two weeks either in the field or in the laboratory (Graham et al., 2013; Nitschke et al., 2016; Ali et al., 2019; Williamson et al., 2021). These contrasting results likely reflect a host-dependent ability to procure symbionts, and/or the differences in symbiont reservoirs and environmental conditions (light, temperature, etc.) among studies.

Increased temperature directly impeded the onset of symbiosis in *P. daedalea* recruits, and this finding was in accordance with most previous studies showing that elevated temperatures greatly impaired the symbiont uptake in coral larvae and recruits (Abrego et al., 2012; Schnitzler et al., 2012; Cumbo et al., 2018; Herrera et al., 2020; Sun et al., 2020; Williamson et al., 2021). The onset of symbiosis in early stages of corals involves a series of complex metabolic adjustment and molecular pathways of both coral and symbionts. For coral host, suppression of mitochondrial metabolism, digestive enzymes and immune functions, arrest in phagosome and upregulation of symbiont recognition and endocytosis pathways have been observed during initial interaction (Mohamed et al., 2016). From the perspective of algal symbionts, alterations in flagellar genes and glycan processing genes occur as a result of a transition from free-living to symbiotic lifestyle (Mohamed et al., 2020). It is possible that elevated temperature may stimulate coral metabolism and/or disrupt these aforementioned molecular signatures, thus impeding symbiont infection process. Moreover, the sensitivity of symbiont infection to thermal stress is highly variable and species-specific. For instance, symbiont uptake rate of *P. daedalea* recruits only declined by 50% at 31 °C relative to 29 °C. In contrast, a 2– 3 °C increase in seawater temperature reduced the proportion of pigmented juveniles by 60 – 90% and 50–70% in *A. millepora* and *A. tenuis*, respectively (Abrego et al., 2012), while the + 3 °C treatment totally arrested the successful symbiont uptake in *A. intermedia* (Sun et al., 2020). It appears that primary symbiont acquisition in the massive coral *P. daedalea* might be more resistant to increasing seawater temperature than *Acropora* species.

Exposure to high pCO_2 had no detectable effect on symbiont uptake success in *P. daedalea*. By contrast, Suwa et al. (2010) found that acidified seawater indirectly delayed symbiont infection in *A. digitifera* primary polyps, mainly due to reduced polyp size under OA and the

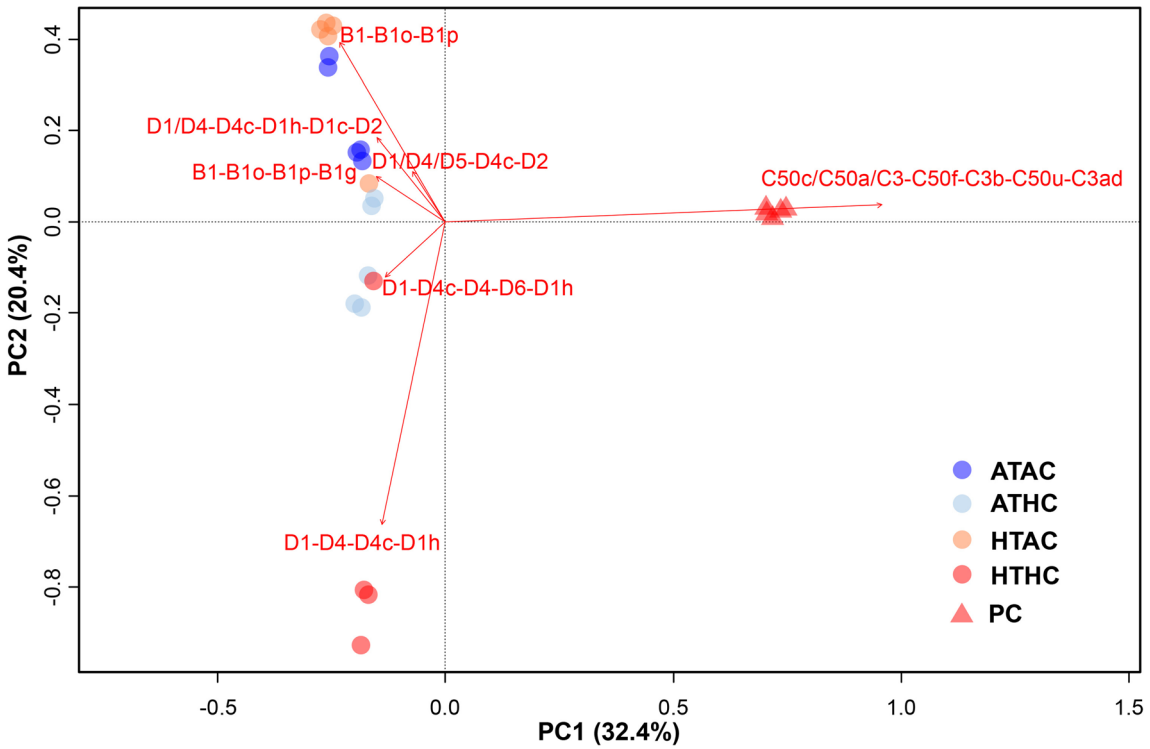

Fig. 5 Principal components analysis (PCA) of *P. daedalea* hosting Symbiodiniaceae subclades under combinations of temperature (29 °C, 31 °C) and pCO_2 ($\sim$ 450 µatm, $\sim$ 1000 µatm) as well as parent colonies (PC)

resultant weakened capacity to produce water currents and capture algal symbionts in the coelenteron. This discrepancy could be explained by two reasons. First, lateral size of juvenile *P. daedalea* was unaffected by OA herein and this may partially explain the similar symbiont infection rates between pCO_2 treatment. Second, differential methodology and approaches may also contribute to this distinction. The study of Suwa et al. (2010) provided juvenile *Acroproa* with high density of freshly isolated Symbiodiniaceae cells from giant clams each day and found that despite a delayed infection process, all juvenile corals became symbiotic at the end of the 4-d inoculation and observation. In our experiment, juvenile corals were left taking up the free-living symbionts in a longer duration, which was more representative of natural infection behavior. Similarly, a recent study also did not find an effect of elevated pCO_2 on symbiosis establishment in juvenile *A. intermedia* in a flow-through experimental system (Sun et al., 2020). Collectively, although these aforementioned studies showed that symbiont uptake process and symbiosis establishment in coral recruits were largely unaffected by high pCO_2, more research on different coral species is still needed.

Juvenile *P. daedalea* harbored a broad range of Symbiodiniaceae ITS2 type profiles, with *Breviolum* (B1-B1o-B1p-B1g and B1-B1o-B1p) and *Durusdinium* (D1/D4-D4c-D1h-D1c-D2) being the most abundant types (82%) at ambient conditions. While these types present in juveniles were not found in adult *P. daedalea*, the dominant type in adults was C50c/C50a/C3-C50f-C3b-C50u-C3ad (90%), indicating flexibility in the type of symbiont initially acquired by corals with a horizontal transmission mode. This finding was in good agreement with the idea that symbionts within larval and juvenile cnidarians are often distinct from adults' symbiont populations before the onset of specificity, which could occur 3.5 years following settlement (Gómez-Cabrera et al., 2008; Abrego et al., 2009b,a; Cumbo et al., 2013; Yamashita et al., 2013, 2014; Ali et al., 2019).

The symbiont community compositions within *P. daedalea* recruits were altered by elevated temperature and pCO_2, and changes in the relative abundance of *Durusdinium* ITS2 type profiles (with D1 as the majority ITS2 sequence) were unique to the treatment group. Surprisingly, the effect of high temperature on the proportion of *Breviolum* was dependent upon the pCO_2 level, and the relative abundance of *Durusdinium* did not increase in *P. daedalea* recruits at high temperatures. Similarly, a most recent study also found that elevated temperature did not affect the proportion of *Durusdinium* in *O. faveolata* recruits, even when they were reared with *Durusdinium* donors (Williamson et al., 2021). These findings contrast to most previous studies demonstrating that *Acropora* recruits preferred to harbor *Durusdinium* spp. (D1 and D1-4) when

exposed to thermal stress (Abrego et al., 2012; Yorifuji et al., 2017; Sun et al., 2020), thus achieving increased heat tolerance in juvenile corals (Mieog et al., 2009; Yuyama et al., 2016). Symbionts of the genus *Durusdinium* are generally considered to be opportunistic and thermally tolerant, and they often live *in hospite* with corals inhabiting warm waters in a broad geographic region globally (Ziegler et al., 2017; Quigley et al., 2018). For instance, *Durusdinium* within *Galaxea fascicularis* in South China Sea occurred most frequently in locations with the highest seawater temperatures (Tong et al., 2017; Zhou et al., 2017). Hence, our results suggest that either not all coral species tend to associate with *Durusdinium* when confronted with thermal stress, or that the specific *Durusdinium* type in the present study may be not optimal for *P. daedalea* to cope with heat stress. Apparently, the identities of coral host and Symbiodiniaceae are both crucial in shaping thermal resilience of coral holobiont.

Intriguingly, high pCO_2 led to a community-wide change in Symbiodiniaceae types. The dominant Symbiodiniaceae types shifted from a combination of *Breviolum* and *Durusdinium* at ambient pCO_2 to the prevalence of *Durusidinium* at high pCO_2. It remains unclear why *Durusdinium* dominated recruits under high pCO_2. A recent study showed that *Durusdinium*-dominated *Platygyra* corals were less likely to bleach but exhibited significantly higher mortality during heat-stress events, compared to those dominated by *Cladocopium* (Claar et al., 2020). Hence, the implications of shifts in Symbiodiniaceae phylotypes within *P. daedalea* at high temperature and pCO_2 for the fitness and resilience of coral recruits warrant further research. We speculate that the varying physiological traits, infectiousness, and proliferation rates of different symbiont phylotypes, together with host selectivity and winnowing process at different temperature and pCO_2, may contribute to the emergence of shifted Symbiodiniaceae community and plasticity of early symbiosis within juvenile *P. daedalea* observed herein (Abrego et al., 2009b, 2012; Quigley et al., 2017). Further comprehensive investigation of fundamental traits of different coral-Symbiodiniaceae associations such as photo-physiology, carbon fixation, and thermal tolerance is needed to explicitly decipher the mechanism underlying Symbiodiniaceae dynamics and symbiosis plasticity in juvenile corals under environmental stress (Abrego et al., 2008; Cantin et al., 2009; McIlroy et al., 2016).

The lateral growth of recruits measured as geometric diameter was significantly reduced by high temperature but not high pCO_2. The decrease in geometric diameter is highly likely a direct result of the delayed symbiont infection success, which suggests that less additional energy via symbiont photosynthesis has been provided to fuel early calcification. The lack of an effect of high pCO_2 on lateral growth of *P. daedalea* recruits was in stark contrast most prior studies showing the detrimental effects of high pCO_2 on the post-settlement linear growth and development of multiple coral species from the genus *Acropora*, *Porites* and *Pocillopora* (Cohen et al., 2009; Albright et al., 2010; de Putron et al., 2010; Albright and Langdon 2011; Jiang et al., 2015, 2018, 2019). The tolerance of juvenile growth to increased pCO_2 in *P. daedalea* is most likely related to its slow calcification rate, which intrinsically implies much less proton production during calcification and therefore less energy cost to export protons and maintain a favorable chemical microenvironment at the calcification site, and this characteristic would largely relieve the energy requirement for calcification under high pCO_2 (Cohen and Holcomb 2009; McCulloch et al., 2012; Comeau et al., 2014).

With respect to survival of *P. daedalea* recruits, our study showed that neither increased temperature, pCO_2, nor their interaction had a remarkable influence. This finding aligns with the majority of previous studies reporting that elevated temperature and/or pCO_2 had no effect on the post-settlement survivorship of juvenile corals (Anlauf et al., 2011; Foster et al., 2015; Jiang et al., 2018; Williamson et al., 2021). Survival likelihood of juvenile corals has been shown to increase with increasing linear growth and colony size (Hughes and Jackson 1985; Babcock 1991). Although declines in linear growth rates have been frequently observed in juvenile corals under elevated temperature and/or pCO_2, the lack of an effect on survival of juvenile corals may simply arise from the fact that most laboratory studies have minimized the key pressures, such as competition, sedimentation, and predation that juvenile corals would suffer in the field. In this case, mortality risk associated with reduced growth rates under environmental stress may become less evident in a laboratory setting. Additionally, the delayed symbiont infection may also be held accountable for the high survivorship at increased temperatures, since algal symbionts constitute a major source of reactive oxidative species, particularly under thermal stress (Yakovleva et al., 2009; Chamberland et al., 2017). The reduction in symbiont infection success in juvenile corals would abate the potential oxidative stress and protect them against cellular damage at high temperatures, thus favoring the survival of recruits. Furthermore, although our temperature treatment was relatively extreme, with an elevation of 2 °C for 4 weeks and an accumulation of 8 DHW, it was still below the recorded ephemeral summer maximum of 33.4 °C on Luhuitou reef (Li et al., 2012; Jiang et al., 2017). Hence, adult *P. daedalea* in our site could have adapted to summer extremes over many years and generations and conferred such tolerance capacity to its offspring.

Taken together, our work suggests that elevated temperature exerted an adverse effect on symbiosis establishment and growth of newly settled *P. daedalea*. However, we found no evidence that elevated pCO_2 impaired symbiont infection, early growth or survival of *P. daedalea* recruits. Furthermore, both increased temperature and pCO_2 affected the Symbiodiniaceae community within coral recruits, reflecting flexibility and plasticity of early symbiosis. Apparently, ocean warming will be more deleterious for the early success and population maintenance of this massive coral species than ocean acidification, potentially retarding the post-settlement development and rendering juvenile corals more susceptible to competition and mortality in the field. Nevertheless, recruits of the massive coral *P. daedalea* appear much more resilient to OW and OA than the well-studied *Acropora*, and therefore climate change may gradually bring about a shift in dynamics and compositions of coral recruits and assemblages, with potential dire repercussions for structural complexity and functional diversity of future reefs.

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Author contributions LJ, GWZ, and HH conceived and designed the experiments; LJ, GWZ, and YYZ performed the experiments; XML, TY, MLG, XCY, JSL, and SL contributed to analysis and materials. LJ and GWZ analyzed the data and wrote the manuscript. All authors commented on the draft and gave final consent for publication.

Declarations

Conflict of interest On behalf of all authors, the corresponding authors state that there is no conflict of interest.

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