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NOTE

Heat-evolved microalgal symbionts increase thermal bleaching tolerance of coral juveniles without a trade-off against growth

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Abstract Global climate change is threatening the persistence of coral reefs as associated summer heatwaves trigger the loss of microalgal endosymbionts (Symbiodiniaceae) from the coral tissues, or coral bleaching. We infected aposymbiotic juveniles of the coral *Acropora tenuis* with either wildtype (WT10) or heat-evolved (SS1 or SS8) Symbiodiniaceae strains *Cladocopium proliferum* (formerly referred to as *Cladocopium goreaui* and *Cladocopium* C1^{acro}). After 10 months at 27 °C, SS8-juveniles were 2 × larger than SS1- or WT10-juveniles. In response to a simulated heatwave (31 °C for 41 days), the WT10-juveniles bleached and showed a decline in respiration while cell densities and respiration in both SS-juvenile groups remained unchanged compared to the controls. These results reveal that some heat-evolved strains can increase the bleaching tolerance of juvenile corals without a trade-off against growth. This response is opposite to the lower nutrient provisioning often reported for naturally thermotolerant Symbiodiniaceae (e.g. genus *Durusdinium*), thereby offering enhanced fitness to the host without the ecological consequences of diminished growth.

Keywords Assisted evolution · Experimental evolution · Coral reefs · Restoration · Symbiosis · Carbon assimilation

Introduction

Reef-building corals are suffering unprecedented mortality caused by the increase in the frequency and intensity of marine heatwaves (Heron et al. 2017). These mass mortality events are caused by coral bleaching, the loss of Symbiodiniaceae from the coral tissue (Weis 2008). Under ambient conditions, Symbiodiniaceae share photosynthates with their coral hosts, fulfilling most of their nutritional requirements (Yellowlees et al. 2008). However, when corals are heat-stressed, damage to the Symbiodiniaceae photosystems (Weis 2008) results in the excess production of reactive oxygen species which is often followed by the loss of Symbiodiniaceae, coral starvation and ultimately mortality if temperatures remain elevated for too long (Wiedenmann et al. 2013; Rädecker et al. 2021). Future projections suggest that even under a 1.5 °C warming scenario, coral reefs will decline by 70–90% (IPCC 2018). This mounting threat has precipitated the need to identify and establish novel approaches to mitigate current and future reef degradation. These approaches include the enhancement of coral thermal tolerance via bioengineering methods (i.e. assisted evolution (van Oppen et al. 2015)).

Experimental evolution of Symbiodiniaceae across replicate cultures and strains can increase in vitro thermal tolerance (Chakravarti and van Oppen 2018; Buerger et al. 2020). Following introduction of heat-evolved coral symbionts of the common and widely distributed species *Cladocopium*

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proliferum (Butler et al. 2023) into *Acropora tenuis* larvae, about a third of the strains increased the thermal bleaching tolerance of larvae (Buerger et al. 2020). Survival was also enhanced at the juvenile phase of *A. tenuis* (Quigley and van Oppen 2022). Here we show enhanced thermal tolerance in *A. tenuis* juveniles infected with heat-evolved *C. proliferum* without a reduction in coral growth at ambient temperature.

Methods

Methods for coral spawning, symbiont inoculation into coral juveniles, heat stress experiment, juvenile size, Symbiodiniaceae cell density, stable isotope incubations, and photosynthetic performance, and respiration are detailed in the Supplementary Information (SI). Heat-evolved (SS1 and SS8) and wildtype (WT10) *C. proliferum* were generated as previously described (Chakravarti and van Oppen 2018; Buerger et al. 2020; SI).

After ~ 10 months of growth, the juveniles were subjected to a heat stress experiment, which started on 10 October 2019 (D0) and lasted for 41 days (4 days of ramping plus 37 days at elevated temperature). Juveniles that were settled onto polystyrene black sheets were transferred to individual, closed plastic containers placed within individual 45 L experimental tanks. These tanks were set at constant flow-through to help maintain the temperature within each container. Three replicate tanks were used for each temperature treatment (27 °C or 31 °C), with $n = 1$–2 replicate containers per strain, with multiple juveniles in each. The position of the containers within each tank was randomised and individual air lines were supplied. HOBO pendant temperature loggers were added to each tank and Osram brand T5 lights (HO 24 W/865) set at 60 PAR (µmol m^{-2} s^{-1}) were positioned above each. Light intensity was confirmed with a ULM-500 light meter with a US-SQS/L sensor (Walz). Temperature in the heat treatment was ramped from 27 to 31 °C at a rate of 1 °C per day, and measurements (survival, growth, symbiont cell densities, photophysiology, respiration, nutrient transfer) started once the heat stress treatment reached 31 °C (15 October 2019: D5). Measurements were then taken at the following timepoints: day 5 (15 October), and days 38, 39, 40, 41 (between 17 and 20 of November 2019). Measurements were staggered over four days given the amount of time each required. Specifically, photographs and PSII maximum quantum yields (F_v/F_m) were collected throughout the heat stress period, stable isotope incubation and cell counts were carried out on days 5 and 39, and respirometry on days 40–41. Physiological and metabolic measurements were performed at similar times of the day to avoid biases linked to circadian rhythms. Symbiont cell densities in the juvenile corals and symbiont photophysiology were used as proxies for bleaching. Other traits, like chlorophyll a, were not measured given the young age of the juveniles and therefore, low biomass available.

All statistical analyses were run in R version v.3.2.3 (R Core Team 2013). Values were calculated as percentage change in the trait between the first (D5) and the final (D41) day of heating. Differences between treatments were assessed using linear models using the nlme package (Pinheiro et al. 2014) with interactive fixed effects of culture (WT10, SS1, SS8) × temperature (27 °C or 31 °C).

Results and discussion

SS-juveniles grow as fast or faster than WT-juveniles at ambient temperature

Symbiodiniaceae exhibit a high level of physiological diversity, with naturally thermotolerant strains typically translocating less carbon to their host at ambient temperatures compared with thermosensitive symbionts, translating to slower host growth (Cantin et al. 2009). To explore whether this trait trade-off has evolved during the experimental heat selection of the Symbiodiniaceae strains, coral juveniles infected with either SS1, SS8 or WT10 derived from the same clonal culture of *Cladocopium proliferum* were maintained at ambient conditions for 10 months at which time their size was measured. Contrary to our expectations, the SS8-juveniles were twice as large (~ 3.6 mm^2) as juveniles infected with SS1 or WT10 (both ~ 1.7 mm^2, lm, $p < 1e\text{-}07$; Fig. 1).

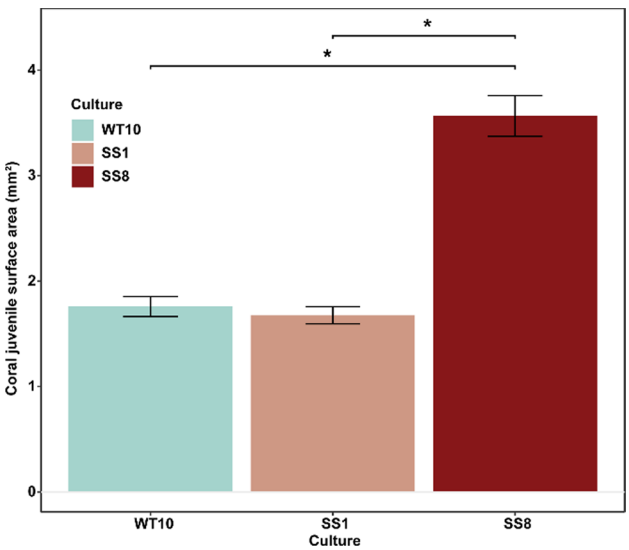

Fig. 1 Surface area ($mm.^2 \pm SE$) of individual coral juveniles infected with *Cladocopium proliferum* strains WT10, SS1, and SS8 ($n = 140$, 174, 242 in each treatment, respectively) after ~ 10 months under ambient conditions (27 °C). Asterisks (*) denote significant differences (lm, $p < 0.05$)

SS-juveniles are more bleaching tolerant than WT-juveniles

We have previously shown that SS1 and SS8 enhance thermal bleaching tolerance (Buerger et al. 2020) and survival (Quigley and van Oppen 2022) of *A. tenuis* larvae or juveniles but the trade-off with growth was unassessed. We therefore subjected the three host-symbiont pairs to a simulated heatwave at a maximum temperature of 31 °C that lasted for 40 days, with a subset of the juveniles kept at 27 °C as controls. To measure the extent of bleaching, the *in hospite* symbiont cell densities in the three host-symbiont pairs exposed to elevated or ambient temperatures were assessed at day 5 and 39 (D5 and D39). When the maximum temperature was reached on D5, the average symbiont cell density in *A. tenuis* juveniles did not differ between temperature treatments for any of the host-symbiont pairs nor among host-symbiont pairs within a temperature treatment (Fig. 2, average across the three ~ 50,000 ± 10,000 cells/mm^2 at 27 °C and ~ 36,000 ± 6,000 at 31 °C). By D39, cell densities at 27 °C had increased slightly for WT10-juveniles and decreased slightly for the SS-juveniles, however, these changes were not statistically significant ($p = 0.19–1.0$ for all pairwise comparisons for differences within timepoint).

At D39, however, symbiont cell densities in WT10-juveniles were 2.6-times lower at 31 °C compared to 27 °C (linear model (lm), $p = 0.0438$), implying that WT10-juveniles lost cells in this timepoint in response to the elevated temperature treatment. In contrast, SS-juveniles (SS1- and SS8-juveniles) showed no difference in Symbiodiniaceae densities at 31 °C relative to 27 °C nor between temperature treatments on D39 ($p = 0.49–1.0$ for all pairwise comparisons). This confirms previous independent results that these heat-evolved symbionts confer increased tolerance to both the larval (Buerger et al. 2020) and juvenile (Quigley and van Oppen 2022) life stages of this coral species.

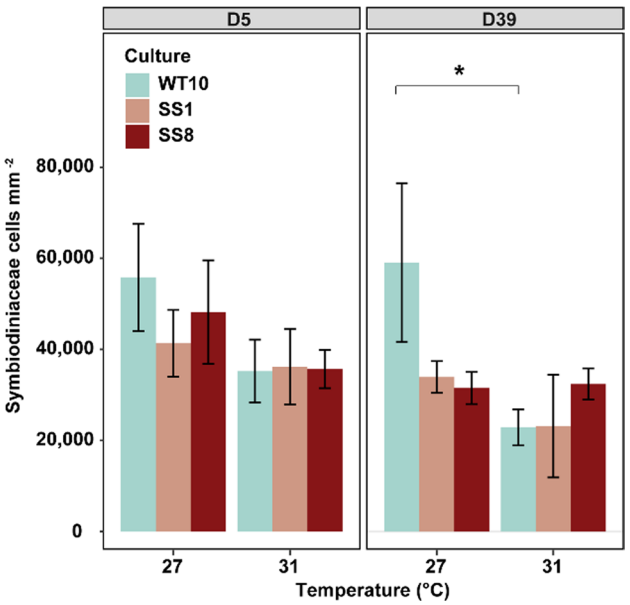

Fig. 2 Symbiodiniaceae cells per surface area of the juveniles (cells/mm^2 ± SE) when the elevated temperature treatment reached 31 °C (D5; $n = 5$ juveniles) and on D39 ($n = 4–6$ juveniles). Asterisks (*) denote significant differences (lm, $p < 0.05$) between temperature treatments for the same strain

The photochemical efficiency of the Symbiodiniaceae photosystem II (maximum quantum yield, F_v/F_m) tends to decline when corals experience heat stress (Ferrier-Pagès et al. 2010) and this measure is therefore commonly used to assess thermal stress in corals (Cantin et al. 2009; Wiedenmann et al. 2013; Buerger et al. 2020). F_v/F_m values were unusually low over the course of the experiment across all juvenile treatments and at both temperatures (0.1–0.3, Fig. S1, while values for healthy corals are typically larger than 0.5 (Suggett et al. 2015)) and this trait was therefore not analysed further. Lower and variable PAM values relative to adults have previously been observed in young juvenile corals inoculated with cultured symbionts and may be indicative of early onset of symbiosis of individual or specific strains (Quigley et al. 2017; Brunner et al. 2022) (Figure S1).

As an additional measure of stress, we assessed dark respiration on D5 and at the end of the experiment (D40–41) (Fig. S2). Overall, respiration was low. On D5, there was no significant difference in O_2 consumption between the 27 °C and 31 °C treatment for any of the three juvenile groups, nor between any pair of juvenile groups at each temperature (Fig. S2A), which may have been due to the small sample size and high level of variation observed. Despite the low experimental replication, WT10-juveniles exhibited higher respiration compared to SS8-juveniles at the ambient temperature on D40–41 ($p = 0.03$), while there was no significant difference in respiration between SS1- and SS8- juveniles ($p = 0.48$). In addition, although respiration at 31 °C compared to 27 °C was slightly lower for WT10-juveniles at the final timepoint, this difference was not statistically significant ($p = 0.12$). There was also no significant difference detected in the SS-juveniles ($p = 0.86$ and 0.99 for SS1- and SS8-juveniles, respectively). We hypothesise that the reduced respiration at elevated temperature can be ascribed to their loss of symbionts. The large changes in respiration for WT10-juveniles occurred at the same time as the largest decrease in symbiont cell densities (2.6-times decrease in density) in the juveniles at D39.

Enhanced tolerance to elevated temperature of SS-juveniles does not come at a cost of reduced growth rate at ambient temperature

Growth, as measured in percentage change in surface area, was not only measured prior to, but also during the 41-day

long heatwave experiment. The growth of the coral juveniles did not differ statistically between ambient and elevated temperatures (Fig. 3A), or among juveniles harbouring SS1, SS8 or WT10 at either 27 °C or 31 °C (lm, $p = 0.162–1$). Our 41 day-long experiment may have been too short to reveal differences in growth between small coral juveniles. Survival was also measured over the course of the heatwave experiment, but there were no statistical differences among the three groups at ambient or elevated temperature, nor between ambient and elevated temperatures for each of the three groups (Fig. 3B; lm, $p = 0.5–0.7$).

To quantify the nutrient fluxes between Symbiodiniaceae and their hosts, coral juveniles were co-incubated with stable isotope tracers. Levels of ^{13}C enrichment were very low and could not be interpreted confidently (Supplementary Results, Fig. S3), but ^{15}N enrichment (via assimilation of ammonium) was high and heterogeneously distributed between host cells and algal symbionts (Fig. 4). No clear trend emerged from the ^{15}N enrichment in the Symbiodiniaceae cells. Indeed, on D5 at ambient temperature, WT10 Symbiodiniaceae were significantly more enriched in ^{15}N than SS08 (Fig. 4A; lm, $p < 0.05$), but this trend was reversed at 31° C, when SS08 symbionts were 38.6% more enriched than WT10 (Fig. 4A; lm, $p < 0.05$). Further, at the end of the experiment (D39), the host tissue adjacent to the algal symbionts was 36.3 to 39.1% more enriched in SS01 and SS08 compared to WT10, at both ambient and elevated temperatures (Fig. 4B, lm, $p < 0.05$). As the Symbiodiniaceae themselves assimilated approximately three-times less nitrogen than these gastrodermal hotspots, it is unlikely the heat-evolved strains translocated more nitrogen to the host tissues and other physiological or microbial processes may be responsible for these differences.

The heat-evolved *Cladocopium* strains provided measurable benefits to the coral holobiont under ambient and elevated temperatures, including faster growth under long-term ambient conditions (SS8-juveniles), the maintenance of symbiont cell densities in juveniles under heat stress and equivalent respiration under ambient and elevated temperatures (SS1- and SS8-juveniles). These patterns confirm previous results with heat-evolved strains (Buerger et al 2022; Quigley and van Oppen 2022). Our results suggest that heat-evolved Symbiodiniaceae can enhance the tolerance of *Acropora* coral juveniles without a trade-off in growth. Importantly, this contrasts with the traits previously described for naturally thermotolerant Symbiodiniaceae in the genus *Durusdinium,* with important implications for ecosystem health. Symbioses of *Acropora* corals with *Durusdinium* are characterised by comparatively high thermal tolerance but low symbiont-to-host carbon translocation and growth at ambient temperature compared with heat-sensitive *Cladocopium* (Cantin et al. 2009). In contrast, *Pocillopora* corals in the far eastern Pacific harbouring *Durusdinium glynnii* have higher thermal tolerance compared with conspecifics that house *Cladocopium latusorum*, but show no metabolic trade-off (Turnham et al. 2023). *Durusdinium* has recently been introduced into the Caribbean (Thornhill et al. 2014), and become more prevalent and abundant over time in some regions possibly due to increasing anthropogenic stressors (Pettay et al. 2015). An increase in *Durusdinium* abundance in Caribbean corals is concerning, specifically due to the early evidence of the trade-off between thermal tolerance and growth in this genus (but see Turnham et al. 2023), which, when modelled, negatively impact resilience and long-term recovery from decreased growth (Ortiz et al. 2013). These results suggest that the experimental evolution

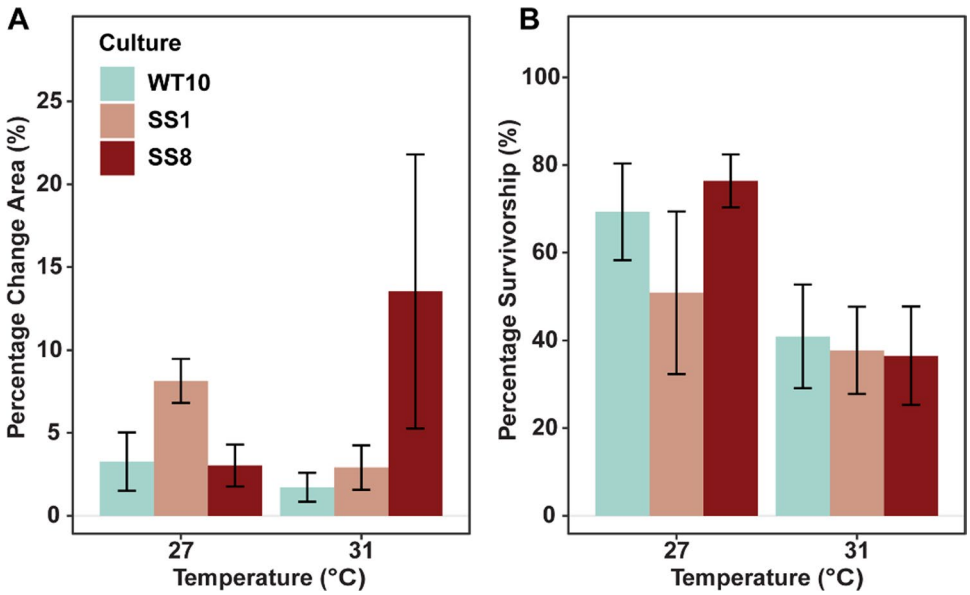

Fig. 3 Fitness traits of *A. tenuis* juveniles infected with *Cladocopium proliferum* strains WT10, SS1 or SS8 over the course of the heat stress experiment at ambient and elevated temperature treatments. **A** Percentage change in surface area (± SE, $n = 18$- 46 juveniles per treatment combination) between the start of the heat stress experiment and D38 and **B** percentage of survival (± SE, $n = 4$ juveniles per treatment combination)

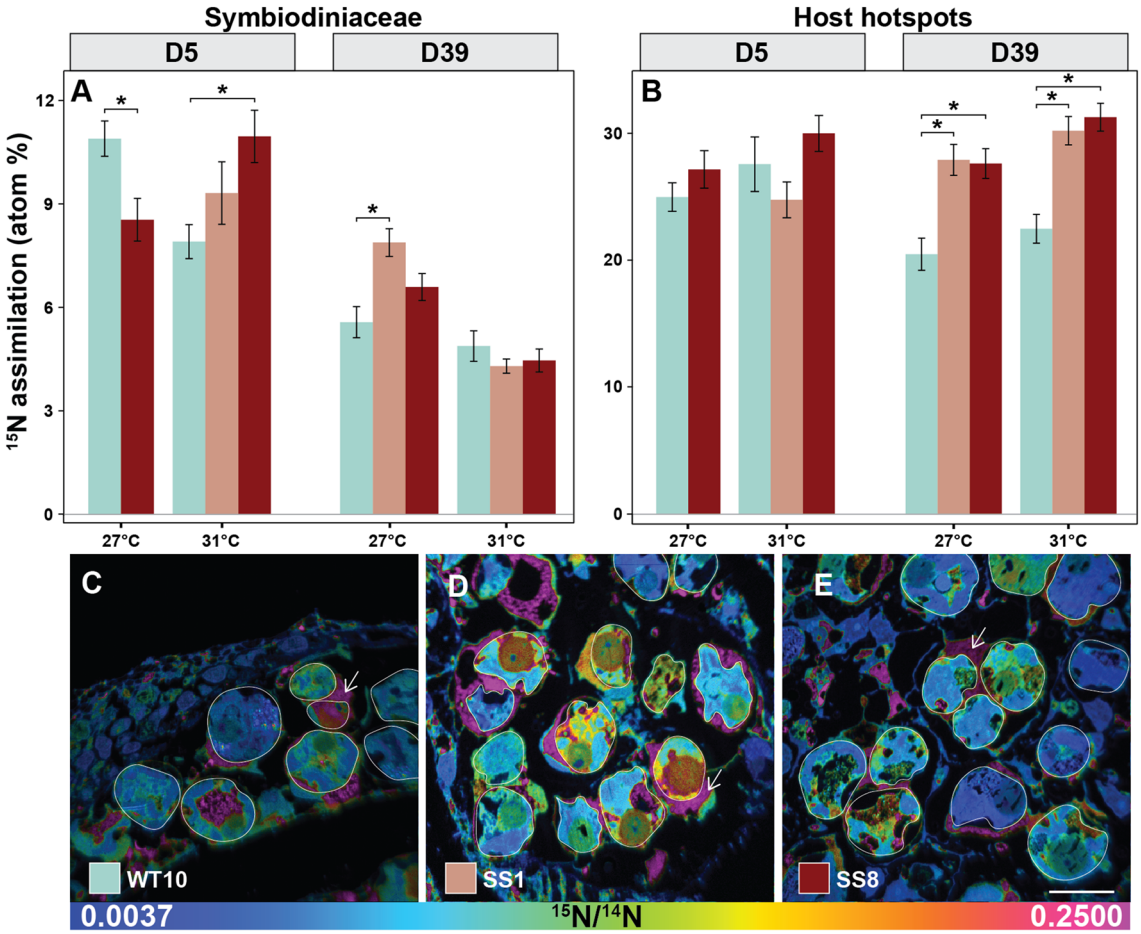

Fig. 4 Nitrogen assimilation (atom % ± SE, $n = 25$ for Symbiodiniaceae, $n = 47$ for host hotspots) by the juveniles and their algal symbionts. Nitrogen assimilation in (**a**) *in hospite Cladocopium proliferum*[o] and **b** hotspots in the gastroderm, within 10 µm from the algal symbionts, during the heat stress experiment. Asterisks (*) denote significant differences (lm, $p < 0.05$). Note: the scale of the y-axes differs between panels (**a**) and (**b**). Representative nanoscale secondary ion mass spectrometry (NanoSIMS) images showing (**c**–**e**) ^{15}N/^{14}N ratio in the algal symbionts (circled in white), and hotspots (white arrows). The ^{15}N/^{14}N images are displayed as Hue Saturation Intensity (HSI) where the colour scale of the ratio, with natural abundance in blue, changing to pink with increasing ^{15}N levels. Scale bar = 10 µm

of Symbiodiniaceae followed by their reintroduction into coral hosts may provide a valuable tool for reef restoration initiatives.

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Author contributions KQ, CAR, MP, and MvO conceived and designed the experiment; CAR and KQ conducted the experiment, KQ and CAR carried out the data analysis; MP and JBR carried out the sample preparation and NanoSIMS data analysis. KQ, JBR and MvO wrote the manuscript. All authors revised and approved the manuscript.

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Data availability All code and data will be available via Github upon publication.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval Corals were collected under the following Great Barrier Reef Marine Park permit to AIMS G12/ 35236.1

Consent for publication All authors consent to publication.

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