Individual variation in growth and physiology of symbionts in response to temperature

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Abstract

In many cases, understanding species level responses to climate change requires understanding variation among individuals in response to such change. For species with strong symbiotic relationships, such as many coral reef species, genetic variation in symbiont responses to temperature may affect the response to increased ocean temperatures. To assess variation among symbiont genotypes, we examined the population dynamics and physiological responses of genotypes of *Breviolum antillogorgium* in response to increased temperature. We found broad temperature tolerance across genotypes, with all genotypes showing positive growth at 26, 30, and 32° C. Genotypes differed in the magnitude of the response of growth rate and carrying capacity to increasing temperature, suggesting that natural selection could favor different genotypes at different temperatures. However, the historical temperature at which genotypes were reared was not a good predictor of temperature response, suggesting a lack of adaptation to temperature over hundreds of generations. We found increased photosynthetic rates and decreased respiration rates with increasing temperature, and differences in physiology among genotypes, but found no significant differences in the response of different genotypes to temperature. In species with such broad thermal tolerance, selection experiments on symbionts outside of the host may not yield results sufficient for evolutionary rescue from climate change.

Keywords: acclimation, adaptation, climate change, coral reefs, genetic variation, symbiosis

Introduction

As anthropogenic changes to the planet increasingly threaten ecosystems, species can respond in four ways (Hughes et al. 2000, Williams et al. 2008). They can migrate to new habitats that resemble the environmental conditions to which they are adapted (Wallingford et al. 2020). Individuals and populations can acclimate to changing environments via phenotypic plasticity in traits that allow them to persist in the same habitat (Williams et al. 2008). Genetic changes in populations can lead to adaptation to changing environments if driven by natural selection on individuals with heritable traits that confer greater fitness in the new environment (Gomulkiewicz and Holt 1995; Carlson et al. 2014). Populations that are strongly affected by a changing environment, but unable to respond in any of these ways, will go extinct (Hughes 2000).

The nature of the response of species to anthropogenic change may depend on the extent of genetic variation in traits among individuals within a species. For many reasons, within species variation can be as important for determining ecological outcomes as variation among species (Des Roches et al. 2018). Rapid evolution that can alter ecological outcomes requires genetic variation upon which natural selection can act (Thompson 1998; Hairston et al. 2005). Increases in genetic diversity can lead to greater stability, resistance to disturbance, and increases in ecosystem function (Hughes and Stachowicz 2004; Schweitzer et al. 2011). Individual variation can allow the broader population to fill more niche space—often species described as generalists are composed of a population of specialist individuals (Bolnick et al. 2003). Different plastic responses to environmental change among individuals may ultimately result in acclimation of populations to various environments. Variation among individuals may be critical

for predicting ecological responses to anthropogenic change (Bolnick et al. 2011; Forsman 2014).

Coral reefs around the world are in crisis, due to a number of factors, but chiefly increasing ocean temperatures (Glynn and D'Croz 1990; Brown 1997; Hoegh-Guldberg 1999). When ocean temperatures exceed a threshold, the mutualism between coral reef species and their dinoflagellate algal symbionts breaks down, resulting in coral bleaching. Given the dependence of hosts on photosynthetically-derived carbon from the algae, bleaching often results in the death of the host organism (Glynn and D'Croz 1990; Eakin et al. 2010, 2019). Given sufficient time, coral reef species and their associated symbionts may evolve in response to increased bleaching events, but predictions of severe or total reef loss by 2050, and the consequences for the multitude of species that are associated with reefs, are dire (Heron et al. 2016; van Hooidonk et al. 2016; Hughes et al. 2018; Oliver et al. 2018).

Standing genetic variation among individuals in response to temperature may offer some hope in the face of these worrisome circumstances. The temperature at which bleaching occurs depends largely on the traits of both the host and the algal symbionts (Baird et al. 2009; Quigley et al. 2018). Standing variation in traits that confer thermal tolerance may allow for human-assisted evolution of reef species via artificial selection on symbionts (van Oppen et al. 2015). Acclimation to temperature may occur if hosts are able to swap symbionts with those in the water column that may offer greater benefits at different temperatures (Buddemeier and Fautin 1993; Baker et al. 2004; Berkelmans and van Oppen 2006; Jones et al. 2008). Both of these mechanisms of adaptation and acclimation via symbionts require standing genetic variation in thermal tolerance. There is some evidence that such variation exists on some reefs. Measurements of growth rates of symbionts *in vitro* suggest that there may be sufficient standing

variation in thermal tolerance within species in many cases (Díaz-Almeyda et al. 2017; Grégoire et al. 2017; Bayliss et al. 2019; Pelosi et al. 2021). Symbiont genotypes from historically warmer reefs can allow for higher growth rates of hosts at higher temperatures (Howells et al. 2012). There is also emerging evidence that symbiont evolution in response to increased temperature can reduce bleaching (Zilber-Rosenberg and Rosenberg 2008; Chakravarti et al. 2017; Chakravarti and van Oppen 2018).

Here we quantified the population dynamics and physiology of several genotypes of a single species of algal symbiont in response to increasing temperature to examine genetic variation in thermal tolerance. In a previous study, we found broad thermal tolerance in growth rate and host survival of genotypes of *Breviolum antillogorgium* up to 30°C (Pelosi et al. 2021). Here we exposed these same genotypes to temperatures up to 32°C while also measuring several traits likely to affect the strength of the mutualism with the host, including growth rate, maximum sustainable population size, photosynthetic rate, and respiration rate.

Methods

We collected symbionts from octocoral colonies in the genus *Antillogorgia* from Elbow Reef and Pickles Reef in the Florida Keys in September 2016 (11-18 m depth). Details of collection and genotyping are available in Pelosi et al. (2021). Briefly, we recovered five unique genotypes of *Breviolum antillogorgium* from the heterogenous mixture of strains within the colonies. Three of these genotypes (G1, G2, G3) were isolated and reared at 26° C for five years (~650-700 generations) prior to the start of the experiment; the other two genotypes (G4, G5) were isolated and subsequently reared at 30° C for the same period of time. We confirmed the presence of a single algal genotype in each culture, but cultures were not axenic with respect to

97 bacteria, archaea, and fungi, which we did not quantify. All cultures were maintained in identical
98 growth chambers and transferred to fresh f/2 media monthly.

99 In March 2021, we initiated a laboratory experiment to measure population dynamics and
100 physiology of each genotype at three different temperatures. We used the stock cultures of each
101 genotype to initiate 15 new replicate 50mL cultures at an initial density of 10,000 cells/mL. Five
102 of these cultures of each genotype were maintained in a growth chamber set at 26° C (actual
103 mean temperature +/- s.d. determined by HOBO Data Logger: 25.5°C +/- 0.51). Another five
104 cultures of each genotype were grown in identical growth chambers set at 30°C (30.1° +/- 0.28)
105 and 32° C (31.6° +/- 0.23). Lights were set on a 12:12 day:night cycle, with average day
106 illumination of 4244 Lux (approximately 59 μmole m^{-2} s^{-1} based on a conversion of 1 lux =
107 0.014 μmole m^{-2} s^{-1}) .

108 Every three days, we removed 50 μL from each culture and performed four replicate
109 hemacytometer counts and used the mean as an estimate of cell density. We estimated densities
110 over time for 34 days, by which time, all cultures had peaked in density and had begun to decline
111 in density. Due to a scheduling error, the cultures grown at 30° C were not counted on day 19.
112 We used the time series data up to the time of maximum density in each culture to estimate per-
113 capita growth rate (r) and carrying capacity (K) using the 'growthrates' package (Petzoldt 2019)
114 in R v. 4.0.2 (R Core Team 2021).

115 On Day 27 of the experiment, we removed two 2 mL from each culture grown at 26° C
116 and used these samples to estimate rates of photosynthesis and respiration at each temperature.
117 Replicate samples were placed in randomly assigned wells in a microrespirometry plate that
118 quantified changes in oxygen concentrations over time (Loligo Systems, Viborg, Denmark) in
119 the 26° C growth chamber. We also placed sterile f/2 samples in two wells in each plate to

account for any background changes in oxygen concentration. Samples were dark-adapted for 10 min before measuring oxygen levels in each well every 15 sec for 10 min. Following this, we turned the lights on in the growth chambers, allowed samples two minutes to acclimate, and then again measured oxygen levels every 15 sec for 10 min. The microrespirometry plates were then moved to the 30° C growth chamber, and later the 32° C growth chamber, and allowed to acclimate for 15 minutes in each growth chamber before measurements were taken. We estimated respiration as the slope of a linear fit to declining oxygen levels over time in the dark, subtracting any background changes in oxygen. Similarly, we estimated net photosynthesis as the slope of a linear fit to increasing oxygen levels, accounting for background changes in oxygen in the light. We estimated gross productivity by adding the absolute value of respiration in each culture to net photosynthesis. We standardized respiration, net photosynthesis, and gross photosynthesis by the number of cells in each well, determined by replicate hemacytometer counts, as above. We used the mean of the two replicate measurements of respiration and photosynthesis for each culture as the estimate for each culture.

At the end of the experiment, samples of each replicate were preserved in 95% ethanol. DNA was extracted and amplified following the methods in Pelosi et al. (2021) to verify symbiont genotype at the end of the experiment. We used Analysis of Variance (ANOVA) to determine the fixed effects of temperature, algal genotype, and their interaction on maximum growth rate (r), carrying capacity (K), respiration, gross photosynthesis, and net photosynthesis in separate tests. All data were visually inspected for normality and heteroscedasticity using Q-Q plots and plots of residuals against fitted values. All data met the assumptions of ANOVA, except for r, which was log-transformed to meet assumptions. All analyses were conducted in the R Statistical Computing Platform (v. 4.0.2).

Results

At the end of the experiment, genetic analyses indicated that each culture contained only the genotype we expected, except for cultures of G3, which contained both G1 and G3. The extent of contamination suggests that the stock culture of G3 likely contained both G1 and G3. We do not know the relative abundances of genotypes in these cultures, so observed results may be driven largely by either G1 or G3, or a combination of the two. Here we present the results for all cultures but note that the results from G3 should be interpreted with caution.

All genotypes had positive growth rates at each temperature. However, different genotypes had different growth rate responses to increasing temperature (Temperature*Genotype: $F_{8,60} = 2.51$, $P = 0.020$). Although every genotype experienced the highest mean growth rate at 30° C, the extent to which growth rates dropped at 32° C varied among genotypes. For example, G1 and G4 showed a steep decline in growth rate at the highest temperature, but G2 and G3 showed little decline (Fig. 1A). Similarly, the extent to which growth rate increased between 26° C and 30° C varied among genotypes, with sharp increases observed for G1, G4, and G5, but little difference observed for G2 and G3 (Fig. 1A). Although carrying capacity tended to be highest at 26° C, we observed more variable responses among genotypes in the response of carrying capacity to temperature (Temperature*Genotype: $F_{8,60} = 8.30$, $P < 0.001$). Some genotypes (G3 and G4) showed a steady decline in K with increasing temperature (Fig. 1B). G1 showed a peak in K at 30° C, G1 and G2 showed a decrease in K at 32° C, but G5 showed relatively little variation with temperature (Fig. 1B).

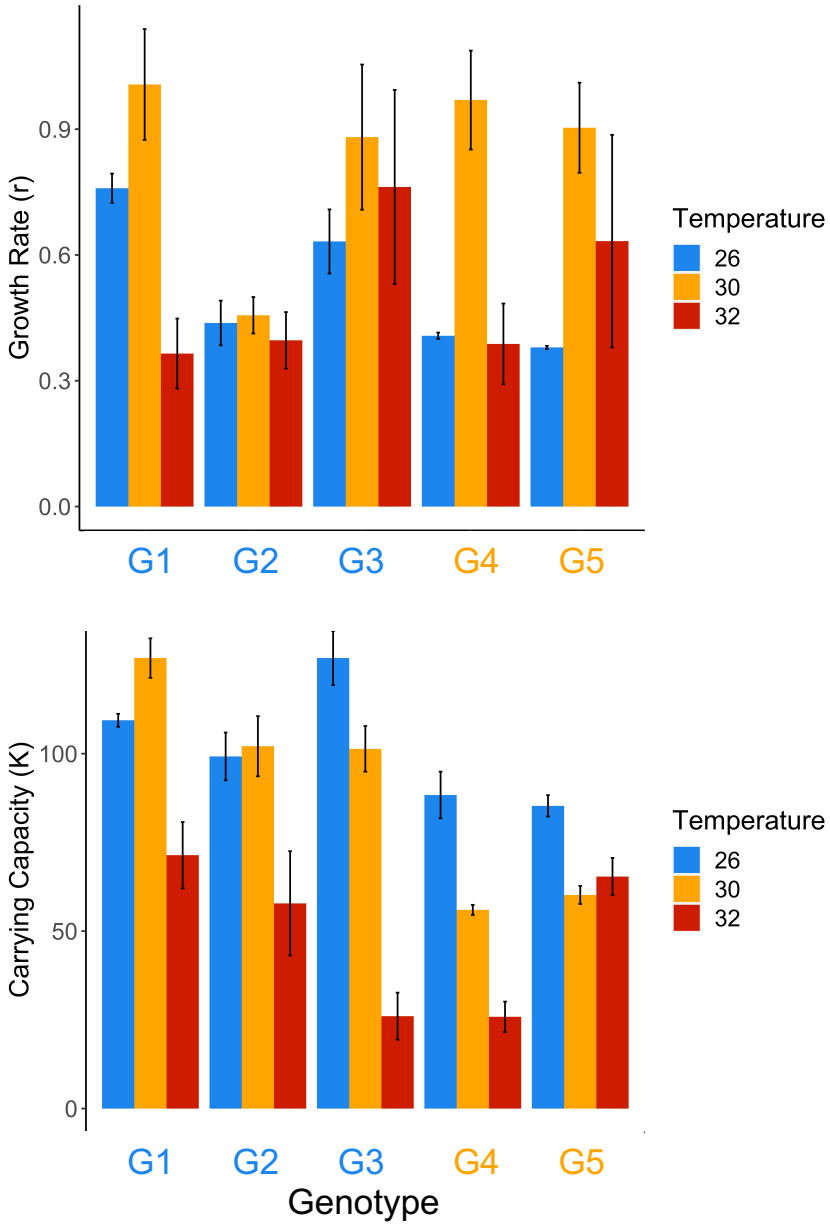

Figure 1. Mean (+/- SE) growth rate (A) and carrying capacity (B) of five genotypes of *Breviolum antillogorgium* grown at three temperatures. Genotypes in blue (G1-G3) were isolated and grown at 26°C and genotypes in orange (G4-G5) were isolated and grown at 30°C.

All genotypes demonstrated the ability to acclimate their physiology to each temperature to some extent, but these responses did not reveal a strong effect of historical temperature. Genotypes had different respiration responses at different temperatures (Temperature*Genotype: $F_{8,59} = 5.82$, $P < 0.001$). One genotype (G5) showed little variation in respiration across

temperatures. The remaining four genotypes showed decreases in respiration at higher temperatures (30° and 32° C) relative to 26° C, but the magnitude of the decrease varied among genotypes (Fig. 2A). Temperature also had a significant effect on gross photosynthesis ($F_{2,59}$ = 6.78, P = 0.002), and genotypes differed in gross photosynthetic rate ($F_{4,59}$ = 24.7, P < 0.001), but, in contrast to the respiration results, there was no significant difference among genotypes in response to increasing temperature (Temperature*Genotype: $F_{8,59}$ = 0.648, P = 0.734). Gross photosynthetic rate increased with increasing temperature, and genotypes G2, G4, and G5 tended to have higher photosynthetic rates than G1 and G3 (Fig. 2B). Patterns of net photosynthetic rate were similar to those for gross photosynthesis; temperature ($F_{2,60}$ = 20.5, P < 0.001) and genotype ($F_{4,60}$ = 31.4, P < 0.001) had a significant effect on net photosynthesis, but again, there was no significant interaction between temperature and genotype ($F_{8,60}$ = 1.12, P = 0.360, Fig. 3).

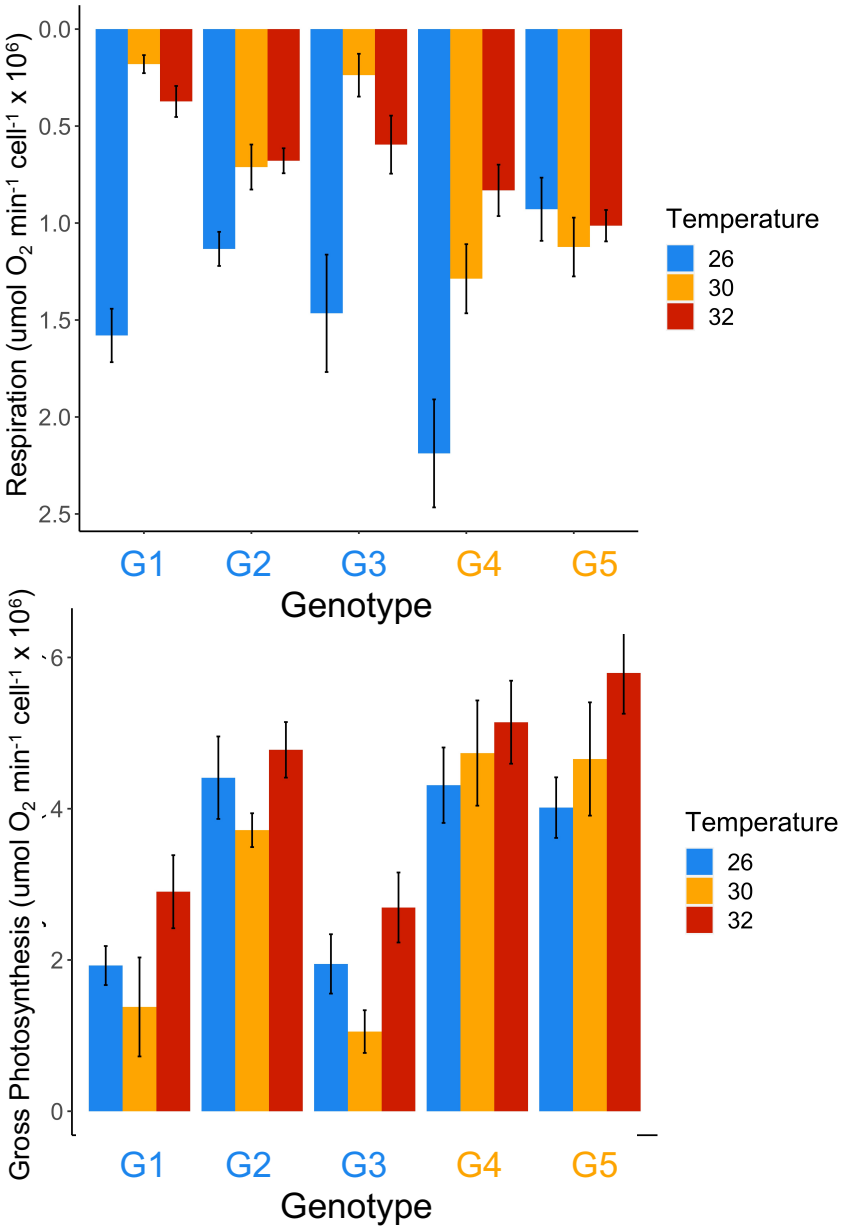

Figure 2. Mean (+/- SE) respiration rate (A) and gross photosynethetic rate (B) of five genotypes of *Breviolum antillogorgium* grown at three temperatures. Rates were standardized by cell density. Genotypes in blue (G1-G3) were isolated and grown at 26°C and genotypes in orange (G4-G5) were isolated and grown at 30°C.

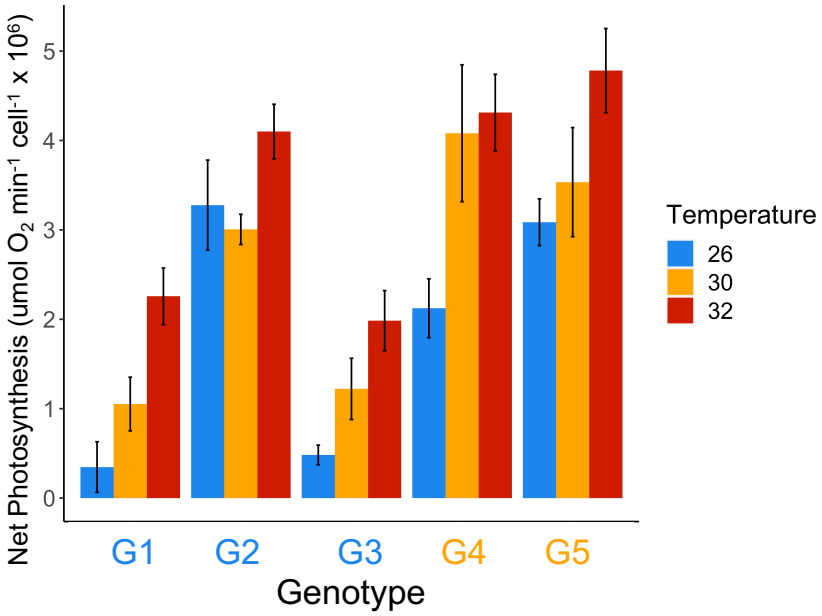

Figure 3. Mean (+/- SE) net photosynthetic rate of five genotypes of *Breviolum antillogorgium* grown at three temperatures, standardized by cell density. Genotypes in blue (G1-G3) were isolated and grown at 26°C and genotypes in orange (G4-G5) were isolated and grown at 30°C.

Discussion

Our results demonstrate broad thermal tolerance in symbionts isolated from *Antillogorgia* octocorals, with positive growth at temperatures up to 32° C, which is beyond the bleaching threshold observed in many coral reef species (Berkelmans 2002). We also observed the highest photosynthetic rates and lowest respiration rates at higher temperatures, suggesting that the potential benefits these symbionts can provide to their mutualist hosts are considerable at temperatures that appear to be stressful to many reef hosts. Population dynamics and physiology were largely dependent on genotype, suggesting that individual variation among symbionts may be important for the strength of mutualism. The lack of difference between historical temperatures suggests that the observed responses are more likely the result of acclimation to different temperatures, rather than evolution in response to historical temperatures.

196 The increased photosynthetic rates we observed with increasing temperature may be
197 explained by increased enzyme activity at higher temperatures, which can be especially
198 important for acclimation to temperature changes (Iglesias-Prieto et al. 1992). An increase in net
199 photosynthesis with increasing temperature suggests that symbionts have more carbon available
200 to provide to the host and may be beneficial across this range of temperatures. Increased
201 respiration rates are often a sign of physiological stress for many organisms, but we generally
202 observed decreased respiration rates with increasing temperature, further indicating broad
203 thermal tolerance. Notably, we measured respiration and photosynthesis at steady state
204 population growth, so these measures may not reflect physiology during exponential population
205 growth. However, as hosts typically regulate symbiont cell densities, physiology during steady
206 state growth may best reflect the strength of mutualism with the host. Although the response of
207 respiration to temperature varied among genotypes, photosynthetic responses to temperature did
208 not. Across species of Symbiodinaceae, respiration and photosynthesis may become decoupled at
209 different temperatures, with respiration often more sensitive to temperature than photosynthesis
210 (Pierangelini et al. 2020). These responses are species-specific, with strong coupling in some
211 species and strong decoupling in other species (Pierangelini et al. 2020). If similar mechanisms
212 occur within species, this may help to explain the different responses of respiration and
213 photosynthesis among genotypes in this study.
214 Although we observed increased growth rates between 26 and 30° C, this is in contrast to
215 Pelosi et al. (2021), where decreased growth rates were observed at 30° C in the same genotypes.
216 We generally did not observe decreased growth rates until 32° C. Potentially, this difference
217 could be driven by the rise of beneficial mutations over the hundreds of generations between
218 studies. An alternative explanation is that these two studies were conducted in different

laboratories (University at Buffalo vs. California State University, Northridge). Although the mean and variance in temperatures were similar laboratories, the light levels in this study were ~60-80% of the intensity of those in the previous study (~60 μmols m^{-2} s^{-1} here, vs. 103.79 to 120.58 μmols m^{-2} s^{-1} in Pelosi et al. 2021). For many photosynthetic organisms, the optimal growth temperature depends on the light environment (Edwards et al. 2016), which may explain the discrepancy between studies. This also highlights the potential importance of light levels in conducting selection experiments in the laboratory and how they may change in natural environments or inside host tissues (Grottoli et al. 2021).

This experiment demonstrates significant differences among algal genotypes, but these differences were observed *in vitro*. These algae spend a portion of their life cycle in the open water and *in vitro* conditions might better mimic this part of the life cycle, although nutrient levels are certainly initially higher in f/2 media than in tropical oceans (Bayliss et al. 2019). The portion of their life cycle of most interest to those studying mutualisms in coral reef ecology is that spent *in hospite*. Whether these *in vitro* differences translate to effects on holobiont responses to temperature remains to be seen. Individual differences in traits in the water column may be important if hosts regularly shuffle symbionts and incorporate new symbionts with different traits from the water column. Because newly settled polyps take up symbionts from the water column, such shuffling occurs at least every generation, although newly settled polyps reduce uptake after 5 months (McIlroy and Coffroth 2017), suggesting limited exchange in adults of this host species. The genotypes in this experiment affected symbiont density at different temperatures in young polyps, but had no effect on polyp survival at increased temperatures (Pelosi et al. 2021). Hosts are likely to regulate symbiont growth rates, maximum density, and

physiology, but the magnitude of this effect relative to the differences among genotypes is unknown.

Our results suggest that each genotype has a different optimum temperature for growth, respiration, and photosynthesis. However, these optima measured *in vitro* may not be the same as what is optimal for the holobiont. When considering a single symbiont species, long-term growth rate of a genotype would be a good proxy for fitness and covariances between traits and fitness should be a good estimate of selection on those traits. However, several studies indicate that symbiont physiologies vary between *in vitro* and *in hospite* (Bhagooli and Hidaka 2003; Ravelo and Conaco 2018; Bellantuono et al. 2019). Thus, when considering evolution in a community context (terHorst et al. 2018), where fitness of the host and associated microbes are tightly linked, selection on the holobiont might not be easily predicted from studies in monocultures. For example, increased symbiont growth rates that are indicative of high fitness *in vitro* or metabolic demand in response to temperature stress might result in increased demand for resources from the host and subsequent breakdown of the mutualism (Rädecker et al. 2021). Although nitrogen is abundant *in vitro*, at least initially, it is likely to be more limiting *in hospite*, with increased nitrogen often destabilizing the mutualism (Rädecker et al. 2015; Morris et al. 2019). The broad availability of nutrients when conducting selection experiments *in vitro* may obscure trade-offs with other algal traits, trade-offs with host traits, or trade-offs with other traits only observed in the context of the holobiont (Chan et al. 2021). Selection experiments on symbionts outside of the host may not yield the evolutionary rescue necessary to adapt to climate change, but rather may require selection experiments on the holobiont.

Conservation-minded assisted evolution for coral reef organisms proposes that selection experiments in the laboratory could yield temperature-tolerant symbiont genotypes that could be

later used to seed reefs experiencing temperature stress (van Oppen et al. 2015). Selection experiments could be conducted on heterogenous cultures composed of standing genetic variation found within and among hosts on reefs (Chan et al. 2021; Pelosi et al. 2021). The genotypes used in this experiment are the result of a long-term selection experiment. Heterogenous cultures of algae were allowed to grow at both 26 and 30° C and the isolated genotypes in this study were unique genotypes that were able to grow well at those temperatures (Pelosi et al. 2021). Additionally, cultures continued to grow at these temperatures for hundreds of generations prior to this experiment and any beneficial mutations would have potentially been subject to positive selection. Nevertheless, we did not observe any obvious effects of historical temperature environment on population dynamics or physiology.

Although our ability to detect an effect of historical temperature was limited to only two genotypes from 30° C and three from 26° C (or two if we consider that G3 was also contaminated with G1), the patterns we observed suggest the opposite of what we would hope for from a successful selection experiment. Genotypes from different evolutionary histories tended to be more similar to each other than genotypes from the same evolutionary history. This suggests that the genotypes recovered from our different selection environments may more likely be the result of genetic drift and random chance than natural selection, or that selection is acting more strongly on traits unrelated to temperature tolerance. As species on coral reefs have repeatedly been exposed to high temperatures in recent decades (Heron et al. 2016; van Hooidonk et al. 2016; Hughes et al. 2018; Oliver et al. 2018), it is possible that these genotypes are already the result of winnowing of non-tolerant genotypes and adaptation to temperature in the ocean, making it difficult to artificially impose further selection. For species such as this that exhibit broad thermal tolerance and are able to acclimate to temperature, selection experiments

may prove more difficult. Whether temperature tolerance results from past adaptation or contemporary acclimatization, hosts that harbor these thermally-tolerant symbionts may have increased resilience to changes in ocean temperatures.

Data Accessibility

Data from this study will be uploaded to Dryad upon acceptance.

Competing Interests

The authors declare no competing interests

Author Contributions

CPt and MAC conceived and designed the experiment and wrote the manuscript. CPt collected and analyzed the data. MAC conducted genetic analyses.

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457 **Figure Legends**

458 Figure 1. Mean (+/- SE) growth rate (A) and carrying capacity (B) of five genotypes of
459 *Breviolum antillogorgium* grown at three temperatures. Genotypes in blue (G1-G3) were isolated
460 and grown at 26°C and genotypes in orange (G4-G5) were isolated and grown at 30°C.
461
462 Figure 2. Mean (+/- SE) respiration rate (A) and gross photosynethetic rate (B) of five genotypes
463 of *Breviolum antillogorgium* grown at three temperatures. Rates were standardized by cell
464 density. Genotypes in blue (G1-G3) were isolated and grown at 26°C and genotypes in orange
465 (G4-G5) were isolated and grown at 30°C.
466
467 Figure 3. Mean (+/- SE) net photosynthetic rate of five genotypes of *Breviolum antillogorgium*
468 grown at three temperatures, standardized by cell density. Genotypes in blue (G1-G3) were
469 isolated and grown at 26°C and genotypes in orange (G4-G5) were isolated and grown at 30°C.