Thermally tolerant symbionts may explain Caribbean octocoral resilience to heat stress

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Abstract

Coral reef ecosystems are under threat from the frequent and severe impacts of anthropogenic climate change, particularly rising sea surface temperatures. The effects of thermal stress may be ameliorated by adaptation and/or acclimation of the host, symbiont, or holobiont (host + symbiont) to increased temperatures. We examined the role of the symbiont in promoting thermal tolerance of the holobiont, using *Antillogorgia bipinnata* (octocoral host) and *Breviolum antillogorgium* (symbiont) as a model system. We identified five distinct genotypes of *B. antillogorgium* from symbiont populations isolated from *A. bipinnata* colonies. Three symbiont genotypes were maintained at 26°C (ambient historical temperature) and two were maintained at 30°C (elevated historical temperature) for two years. We analyzed the *in vitro* growth rate and carrying capacity of each genotype at both ambient and elevated temperatures. All genotypes grew well at both temperature treatments, indicating thermal tolerance among these *B. antillogorgium* genotypes. We also inoculated juvenile *A. bipinnata* polyps with each of the five symbiont genotypes, and reared these polyps at both ambient and elevated temperatures. All genotypes were able to infect polyps at both temperature treatments. Survivorship of polyps at 30°C was significantly lower than survivorship at 26°C, but all treatments had surviving polyps at 56 days post-infection, suggestive of broad-scale thermal tolerance in this system. The widespread thermal tolerance observed in *B. antillogorgium* may play a part in the increased resilience of Caribbean octocorals during heat stress events.

Introduction

In recent years, anthropogenic climate change has severely impacted coral reefs, which comprise some of the most biodiverse and economically important ecosystems on Earth (Hoegh-Guldberg 2011; Hoegh-Guldberg et al. 2017). The success of reef ecosystems in oligotrophic tropical waters is due to their obligate endosymbioses with dinoflagellate algae in the family Symbiodiniaceae (Muscatine and Porter 1977). In this mutualistic relationship, the coral host relies on photosynthate produced by its intracellular symbionts to thrive (Muscatine and Cernichiari 1969; Muscatine and Porter 1977). In exchange for these metabolites, the symbiotic algae recover host nitrogenous waste. This relationship, which is vital to the survival of the reef ecosystem, is currently threatened by anthropogenic environmental changes.

Coral bleaching, or the loss of algal symbionts, occurs due to a variety of environmental stressors including changes in salinity, pH, light intensity and, most commonly, water temperature (Hoegh-Guldberg 1999). Most reef ecosystems now routinely encounter temperatures at or above their thermal maximum each year (Hoegh-Guldberg et al. 2007; Eakin et al. 2009) with bleaching events becoming more frequent and severe (Heron et al. 2016; van Hooidonk et al. 2016; Hughes et al. 2018; Oliver et al. 2018). As ocean temperatures continue to increase, it is essential to understand if and how corals and their symbionts can respond to the present threat.

The holobiont (host + symbiont) can respond to rising seawater temperatures via changes in the symbiont community within a single host, with the basic premise that hosts associating with more thermally tolerant symbionts will be more likely to survive bleaching events (i.e., shuffling and/or switching, *sensu* Baker 2003). Although most coral species host a single dominant symbiont type (Goulet 2006; LaJeunesse 2020), many hosts also harbor background or

70 cryptic symbionts that are often undetected by traditional techniques (Mieog et al. 2007; Correa
71 et al. 2009; Silverstein et al. 2012). During thermal stress, a more thermally tolerant background
72 symbiont can become the dominant symbiont type, potentially conferring thermal tolerance to
73 the holobiont (Berkelmans and van Oppen 2006; Jones et al. 2008; LaJeunesse et al. 2010;
74 Keshavmurthy et al. 2012; Silverstein et al. 2015). In the aforementioned studies, the background
75 symbionts often differed at the genus level and were typically replaced by the previously
76 dominant symbiont type once the stress had subsided (Berkelmans and van Oppen 2006; Jones et
77 al. 2008; LaJeunesse et al. 2010; Keshavmurthy et al. 2012; LaJeunesse 2020).

78 Thermally tolerant symbionts may arise through adaptation of symbiont populations,
79 even in the absence of evolutionary changes in the host. Large population sizes, short generation
80 times, and high mutation rates could greatly augment the standing genetic variation within
81 symbiont populations (van Oppen et al. 2011). This increased variation may allow for selection
82 of particular genotypes in response to elevated sea surface temperatures (van Oppen et al. 2011;
83 Torda et al. 2017; Quigley et al. 2018; Buerger et al. 2020). Numerous studies have established
84 that more thermally tolerant symbionts can be selected from within a single symbiont genus or
85 species (Howells et al. 2011; Chakravarti et al. 2017; Buerger et al. 2020). By sampling
86 symbionts in the genus *Cladocopium* (ITS type C1) from reefs with different temperature
87 regimes, Howells et al. (2011) showed that distinct symbiont genotypes had different thermal
88 tolerances. Symbionts from the warmer reef displayed increased performance and survival at
89 elevated temperatures compared to those from the cooler environment. Furthermore, juvenile
90 hosts inoculated with symbionts from the warmer reef performed better (grew faster, had less
91 bleaching, and had lower mortality) than those inoculated with symbionts from the cooler
92 region.

93 Chakravarti et al. (2017) and Buerger et al. (2020) presented evidence for selection
94 within a species by examining the adaptive capabilities of the symbionts within a monoclonal
95 culture of *Cladocopium goreaui*. Symbionts selected for thermal tolerance (i.e., those maintained
96 at 31°C for 2.5 to 4 years) performed better at 31°C than the original monoclonal culture that had
97 not undergone this selection treatment. Juvenile polyps infected with symbiont cells from the
98 elevated temperature grew at 27°C and 31°C, while polyps infected with the original culture
99 showed negative growth at 31°C. Infection with some strains of thermally selected cells,
100 however, did not reduce the rate or severity of bleaching compared to those infected with the
101 original culture (Buerger et al. 2020). These studies establish that selection for thermal tolerance
102 can occur over ecologically relevant time scales within at least some symbiont populations.
103 Given sufficient standing genetic variation, this is likely the case for other species within
104 Symbiodiniaceae and requires further inquiry (Chakravarti et al. 2017).

105 By starting with a single monoclonal culture, all genetic variation in the symbionts used
106 by Chakravarti et al. (2017) and Buerger et al. (2020) likely arose from *de novo* mutation.
107 Intraspecific markers have revealed numerous instances where host colonies harbor multiple
108 genotypes within a symbiont species (Santos et al. 2003, 2017; Howells et al. 2009; Kirk et al.
109 2009; Pettay and LaJeunesse 2007), suggesting the possibility for selection on pre-existing
110 genetic variation within *in hospite* populations, resulting from both mutation and recombination
111 (Baums et al. 2014; Chi et al. 2014; Wilkinson et al. 2015; Thornhill et al. 2017). Indeed, many
112 studies have found variation in thermal tolerance within a single symbiont species (Karim et al.
113 2015; Díaz-Almeyda et al. 2017; Grégoire et al. 2017; Bayliss et al. 2019). For example, Bayliss
114 et al. (2019) identified genetic and trait variation within and among *Breviolum* species isolated
115 from the octocoral *Antillogorgia bipinnata*. Here we ask if there are thermally tolerant genotypes

present in a symbiont species within a single host colony. We utilized the octocoral, *A. bipinnata* and its host-specialist endosymbiotic algae, *Breviolum antillogorgium* as a model to study the role of symbiont genetic variation in the thermal response of the holobiont. We first selected for thermally tolerant symbionts from *in hospite* populations to capitalize on pre-existing genetic variation. We then examined symbiont growth at two temperatures with an *in vitro* experiment using these cultured isolates of *B. antillogorgium*. Subsequently, we examined whether thermal tolerance in the symbiont could be conferred to the host using an *in hospite* experiment. The presence of thermally tolerant symbionts within a population contained in a single host colony expands the potential for the holobiont to ameliorate the consequences of increased temperatures, potentially slowing the loss of these invaluable ecosystems.

Methods

Symbiont Isolation

Although culturing of Symbiodiniaceae is highly selective and rarely recovers the symbionts dominant within the host (Santos et al. 2001; LaJeunesse 2002), *Breviolum antillogorgium* (ITS2-type B1) has been successfully cultured and associates specifically with octocorals in the genus *Antillogorgia* (Santos et al. 2004; Parkinson et al. 2015). Symbiont cells were isolated from sixteen adult colonies of *A. bipinnata* collected from 12-18 m depth at sites in Elbow and Pickles Reefs, Florida Keys in September 2016 (Table 1). An approximately 2 cm piece was sampled from each colony and homogenized in 2 mL of filtered seawater (FSW). Each homogenate was passed through a series of meshes (250, 120, 70 and 20 µm), followed by several 1 mL 0.22 µm FSW rinses for a final volume of 3-6 mL. Homogenates were spun at 800 rpm for five minutes. Supernatants were decanted, and the pellets resuspended in 10 mL of FSW.

139 This was repeated three additional times, followed by resuspending each pellet in 1 mL of f/2
140 media (Guillard and Ryther 1962). For each colony, 50 µL of the resultant cell suspension was
141 added to six flasks containing 30 mL of f/2 media. Three flasks of the symbionts from each
142 colony were maintained at 26°C, mimicking typical thermal conditions, and three were
143 maintained at 30°C to select for thermally tolerant strains from symbiont populations within a
144 single colony. Initial growth, observed as a small dot at the bottom of the flask, was immediately
145 transferred into a new flask with fresh media. This was repeated each time new growth was
146 observed over the first month, resulting in 131 cultures. All cultures were transferred to fresh
147 media monthly.

148 **Genotype Identification**

149 After two years (~500 asexual generations, assuming an average doubling time of ~1.5
150 days, based on growth rates from this study), total genomic DNA from 106 cultures and the host
151 source colonies was extracted using the 2X CTAB method (Coffroth et al. 1992), diluted to 5-10
152 ng μL^{-1} and the flanking region of the B7Sym15 microsatellite (Pettay and Lajeunesse 2007) was
153 sequenced (TACGEN, Richmond, California, USA). Sequences were compared to available
154 sequences on NCBI using BLASTn for symbiont species-level identification.

155 Cultures identified as *B. antillogorgium* (n=24) were amplified with six microsatellite
156 loci following published protocols: CA 6.38 (Santos et al. 2003), GV_1C (Andras et al. 2009),
157 B7Sym4, B7Sym8, B7Sym34, and B7Sym36 (Pettay and Lajeunesse 2007). Amplicons were
158 labeled with fluorescently tagged primers and visualized on a 6.5% polyacrylamide gel with the
159 LI-COR NEN Global IR2 DNA Sequencer (LI-COR Biotechnology Division) following Santos
160 et al. (2003). Allele sizes were scored by eye relative to size standards between 50 and 350 bp.
161 Based on the microsatellite results, five genetically distinct *B. antillogorgium* cultures (three

162 reared at 26°C and two reared at 30°C) were selected for use in the experiments outlined below
163 (Table 1).

164 ***In vitro* Growth Experiments**

165 Six replicates of each of the five cultures identified above were started by inoculating 18
166 mL of f/2 media with 10,000 cells mL^{-1}. Three of the replicates for each culture were
167 subsequently grown at 25.53 ± 0.89°C (mean ± SD; referred to as "26°C") and three were grown
168 at 29.84 ± 0.48°C (mean ±SD; referred to as "30°C") on a 12:12 h light:dark cycle. Light levels
169 were maintained at 120.58 ± 11.14 $\mu mols m^{-2}s^{-1}$ (mean ±SD) (Philips Energy Advantage T5 HO
170 49W) and 103.79 ± 37.3 $\mu mols m^{-2}s^{-1}$ (mean ±SD) (Philips T5 HO 24W/840) for the 26°C and
171 30°C incubators, respectively (Welch's Two Sample t-test, P = 0.13). Cell densities were
172 measured using six replicate hemocytometer cell counts every three days. Experiments were
173 continued until cell density peaked and exhibited a consistent decline in abundance. We used the
174 'growthrates' package ver. 0.8.1 (Petzoldt 2019) in R ver. 4.0.2 (R Core Team 2020) to fit
175 logistic growth curves to the cell counts over time, up to the maximum cell count for each
176 culture. We extracted values of r and K from the growthrates package and used these as
177 estimates of maximum population growth rate and carrying capacity, respectively. Samples of
178 each replicate were then preserved in 95% ethanol, from which DNA was extracted and
179 amplified following the methods detailed above to verify symbiont genotype at the end of the
180 experiment.

181 ***In hospite* Experiments**

182 Branches from adult colonies of *A. bipinnata,* a gonochoric surface brooder (Kahng et al.
183 2011), were collected from Tennessee Reef, Florida Keys in November 2018 (N 24° 45.150' W

184 81° 45.275'). These branches were transported to flow-through seawater tanks at Keys Marine
185 Laboratory, where they were monitored daily for release of embryos. Brooded larvae were
186 collected from the colony surface on 16, 17, 22, 23, 25, and 26 November, washed three times in
187 0.22 µm filtered instant ocean (FIO), and transferred to Buffalo, NY. Following transport, larvae
188 (4-14 days old) were again washed in FIO to remove environmental contaminants and groups of
189 25 larvae were transferred to pre-conditioned polypropylene containers (volume = approx. 115
190 mL) with 50 mL of FIO. For pre-conditioning, the bottom and sides of each container were
191 roughened with sandpaper and then soaked for four weeks in 1.2 µm filtered seawater (FSW) to
192 establish a biofilm. Once larvae were added, water in the containers (FIO) was changed daily for
193 one week, then every other day until larvae settled and metamorphosed into polyps
194 (approximately 4 weeks). Containers of newly settled polyps (approximately 1-2 weeks post-
195 settlement) were then divided into five treatments (G1 through G5, Table 2) and each treatment
196 was inoculated with 1000 cells mL^{-1} of one of the five genotypes of *B. antillogorgium* utilized in
197 the *in vitro* experiments (Table 1). For each genotype, twelve containers were placed at 26ºC
198 (25.51 ± 1.10°C; mean ±SD) and eight at 30ºC (29.79 ± 0.28°C; mean ±SD) and maintained in a
199 12:12 h light:dark cycle. Initial inoculations occurred twice over a period of one week. Polyp
200 locations in the containers were mapped and survival for each individual polyp was recorded
201 every 3-4 days. As visual infection, indicated by a light brown coloration in the polyp, was not
202 observed within the first month following initial introduction of symbionts, inoculations were
203 then repeated with each water change and combined with feeding with Reefs-Roids (Polyplab,
204 Quebec, Canada) (2 mL of Reef-Roids solution at a concentration of 1 mg/mL FIO) to induce
205 symbiont uptake. Inoculations continued for an additional month, after which the experiment was
206 terminated due to overgrowth by an unidentified green alga which led to extensive polyp

207 mortality at that time. Following the termination of the experiment, surviving polyps were
208 collected, symbiont cell densities were calculated (as detailed above), and all collected polyps
209 were preserved in 95% ethanol. Symbiont DNA was extracted from collected polyps following
210 quantification of cell density and symbiont genotype was verified using the six microsatellite loci
211 described above.

212 **Statistical Analyses**

213 We analyzed the effects of temperature treatment and genotype on *in vitro* symbiont
214 maximum population growth rates and carrying capacity using separate two-way ANOVA,
215 including the interaction term between these factors. Data were log-transformed to meet
216 assumptions of homoscedasticity. In the *in hospite* experiment, we quantified polyp survivorship
217 as the proportion of surviving polyps at the end of one and two months post initial inoculation.
218 We analyzed one and two month survivorship as above; month one survivorship data were
219 arcsine transformed to meet assumptions of normality. We analyzed treatment and genotype
220 effects on symbiont cell densities as above; data were square-root transformed to meet
221 assumptions of normality. Assumptions of normality and homogeneity were checked using
222 residuals and Q-Q probability plots. All post-hoc pairwise comparisons were carried out using
223 Tukey's HSD (honest significance test) (Tukey 1949). *In vitro* statistical analyses were
224 conducted in R ver. 4.0.2 and *in hospite* analyses in R ver. 3.6.1 (R Core Team 2020). R scripts
225 are available at https://github.com/jessiepelosi/symbiont_tolerance and raw data are available at
226 https://www.bco-dmo.org/award/658940.

227 # Results

228 **Sequencing and Genotyping**

229 Following two years of growth at either 26°C or 30°C, there were 131 viable cultures. As
230 noted above, culturing is notoriously selective and often not representative of the dominant
231 symbiont within the host, resulting in the recovery of background or cryptic symbionts (Santos et
232 al. 2001; LaJeunesse 2002; LaJeunesse et al. 2012; Parkinson et al. 2015). This was observed
233 here where the majority of cultures either could not be classified or aligned most closely with *B.*
234 *minutum*, a species commonly recovered from a range of host species where it is undetected by
235 normal techniques (LaJeunesse et al 2012; Parkinson et al 2015). Twenty-four of the cultures
236 were identified as *B. antillogorgium* based on a comparison of B7Sym15 microsatellite flanking
237 region sequences to available sequences on NCBI. Using microsatellite data, we found that 21 of
238 the 24 *B. antillogorgium* cultures (10 reared at 26°C and 11 reared at 30°C) contained a single
239 genotype. Among these 21 cultures, we identified five distinct *B. antillogorgium* genotypes
240 (three reared at 26°C and two reared at 30°C). These five genotypes were used in the *in vitro* and
241 *in hospite* experiments.

242 ***In vitro* growth experiments**

243 Cultures reached their maximum cell density in 28-40 days and maintained steady state
244 growth for at least several days afterward (Fig 2a, Online Resource 1). The effect of temperature
245 on maximum population growth rate was dependent on the symbiont genotype
246 (Temperature*Genotype: $F_{4,20} = 30.2$, $p < 0.001$). At 30°C the mean growth rate for four of the
247 five genotypes decreased from that at 26°C, but to varying magnitudes; the mean growth was
248 slightly higher at 30°C for one genotype (G5) (Fig. 2a). The effect of temperature on carrying
249 capacity also depended on the symbiont genotype (Genotype*Temperature: $F_{4,20} = 17.90$, $p <$
250 0.001). Genotype responses to temperature ranged from a decrease in carrying capacity (G1,
251 G3), to little response (G2, G4), to an increase in carrying capacity (G5) (Fig. 2b). The time for

252 populations to reach their peak abundance was dependent on the interaction between temperature
253 and symbiont genotype (Temperature*Genotype: $F_{4,20} = 6.57$, $p = 0.002$).

254 ***In hospite* polyp experiments**

255 We found a significant interaction between the effects of temperature and genotype on
256 the symbiont cell densities in polyps at the end of the experiment ($F_{4,264} = 6.342$, $p < 0.001$).
257 Average symbiont densities in polyps increased at higher temperature in some genotypes but not
258 others (Table 3, Fig. 3). At one month post-infection, polyp survivorship was not significantly
259 affected by symbiont genotype ($F_{4,85} = 0.64$, $p = 0.636$), nor by treatment temperature ($F_{1,85} =$
260 2.61, $p = 0.110$), and there was not a significant interaction term ($F_{4,85} = 0.673$, $p = 0.613$) (Fig.
261 4a, b). At two months post-infection, survivorship decreased dramatically in both temperature
262 treatments (Fig. 4a, c), with those polyps at 30°C having significantly lower survivorship than
263 those reared at 26°C ($F_{1,72} = 26.90$, $p < 0.001$). There was also a significant effect of symbiont
264 genotype on survivorship at the two-month mark ($F_{4,72} = 2.95$, $p = 0.026$). There was not a
265 significant interaction between temperature and genotype on two-month survival ($F_{4,72} = 0.56$, p
266 $= 0.692$).

267

268 # Discussion

269 Cultured symbionts isolated from standing genetic variation within host colonies revealed
270 the presence of multiple genotypes capable of growth at 30°C, with three of the five unique
271 genotypes used here isolated from a single host colony (Table 1). Genotypes varied in their
272 response to thermal stress (i.e., growth rate and carrying capacity, Fig. 2) and there was no clear
273 evidence of selection for increased thermal tolerance over time in cultures reared at 30°C for

274 over 500 generations (2 years) (Figs. 2-4). However, the ability of all tested genotypes to grow
275 positively at 30°C suggests that *B. antillogorgium,* as a species, is thermally tolerant.

276 ***In vitro* growth experiments**

277 By isolating genotypes that were able to grow at elevated temperatures from standing
278 genetic variation among *in hospite* populations, we established that thermally tolerant symbiont
279 genotypes are present within the host. We maintained these cultures at 30°C for two years to
280 select for long-term growth at elevated temperatures, anticipating that these cultures would adapt
281 and outperform other genotypes at elevated temperature. Unlike previous studies that found
282 evidence for thermal adaptation in Symbiodiniaceae after just one year of exposure to increased
283 temperatures (*Cladocopium* - Chakravarti et al. 2017; *Fugacium, Gerakladium, Symbiodinium* -
284 Chakravarti and van Oppen 2018), long-term growth at 30°C did not yield better performance for
285 *B. antillogorgium* at 30°C (as compared to 26°C). Our results suggest that local adaptation to this
286 temperature did not occur over this time period. Interestingly, growth rates were often counter to
287 our expectations, with G3 (historical thermal regime of 26°C) showing little change in growth
288 rate under increased temperature and G4 (historical thermal regime of 30°C) showing a decrease
289 in growth rate at 30°C compared to growth at 26°C (Fig. 2).

290 While growth rate serves as a good proxy for fitness of symbiont populations, carrying
291 capacity is likely a proxy for efficiency of resource use or metabolite buildup. We found
292 variation among genotypes in carrying capacity, but interestingly, also found variation in the
293 response of carrying capacity to increased temperature. Two of the five genotypes (G2, G5)
294 showed an increase in mean carrying capacity at higher temperature, but one genotype (G3)
295 showed a marked decrease in carrying capacity at higher temperature (Fig. 2b). These
296 differences were unrelated to the temperature at which cultures were historically grown, and the

297 three genotypes isolated from the same host showed the most variation in response (G3, G4, G5).
298 The metabolic theory of ecology and at least one experiment on phytoplankton predict decreases
299 in carrying capacity with increasing temperature (Savage et al. 2004; Bernhardt et al. 2018), but
300 only two of our five genotypes matched this prediction. However, higher metabolic rates should
301 result in increased growth rates. There was a weak positive correlation between r and K ($t = 2.91$,
302 $df = 28$, $P = 0.007$, $r = 0.482$) and these two variables combined to influence the time to reach
303 peak density. Three genotypes (G1, G4, G5) took longer to reach peak density at higher
304 temperature, but the other two genotypes reached peak density more quickly at higher
305 temperature. Although one genotype (G5) with long-term growth at 30°C showed increases in
306 growth rate and carrying capacity at the increased temperature, perhaps reflective of adaptation
307 to increased temperature, this result should be interpreted with caution, as the other genotype
308 with long-term growth at 30°C (G4) did not show the same response (Fig. 2b). The ability of all
309 cultures to survive and grow positively at 30°C for several months, regardless of historical
310 temperature, suggests that these genotypes of *B. antillogorgium* possess pre-existing, broad-scale
311 thermal tolerance, possibly due to plastic acclimatory responses.

312 Prior studies examining the potential for symbionts to adapt to elevated temperatures
313 have focused on a single genotype (Chakravarti et al. 2017; Chakravarti and van Oppen 2018),
314 but in the present study we used several genotypes within the same species, selected from pre-
315 existing genetic variation within *in hospite* populations. Variability in response to elevated
316 temperatures observed among the genotypes used in our experiments highlights the potential role
317 of intraspecific genetic variation in responding to thermal stress and further underscores the
318 importance of examining both intrageneric and intraspecific variation in thermal responses.
319 Other *in vitro* studies (Chakravarti et al. 2017; Chakravarti and van Oppen 2018; Buerger et al.

320 2020) have explored how dinoflagellate symbionts in other genera respond to temperatures
321 higher (31°C-34°C) than used in our experiments. It is possible that the rapid adaptation seen in
322 previous studies might also occur in *B. antillogorgium* if these genotypes were grown at higher
323 temperatures. Further work should explore the limits of thermal tolerance in *B. antillogorgium* to
324 temperatures greater than 30°C as these increasingly elevated temperatures may soon become
325 commonplace on coral reefs (Heron et al. 2016; van Hooidonk et al. 2016; Hughes et al. 2018;
326 Oliver et al. 2018).

327 ***In hospite* polyp experiments**

328 *Antillogorgia bipinnata* polyps were infected by each symbiont genotype at both
329 experimental temperatures. The fact that polyps took up all genotypes is perhaps not surprising,
330 since *B. antillogorgium* is a host-specialist for *Antillogorgia* species (Parkinson et al. 2015), and
331 the symbiont cultures used in this study were initially isolated from *Antillogorgia* host colonies.
332 While Newkirk et al. (2020) found that certain strains of *Breviolum* were unable to establish a
333 stable symbiosis at elevated temperatures with the model scyphozoan *Cassiopeia xamachana*, by
334 focusing our study on a known symbiont of *A. bipinnata*, we demonstrate that the successful
335 establishment of the symbiosis may not always be limited by environmental temperature.

336 Although there were no consistent differences between *in hospite* symbiont densities,
337 final symbiont cell density varied with genotype and temperature, indicating that symbionts were
338 either taken up or grew at different rates within hosts. The lack of a consistent effect of
339 temperature on symbiont density within polyps suggests that the establishment of the symbiosis
340 at 30°C may be as stable as those at ambient temperatures (26°C). Given the significant
341 interaction between treatment temperature and symbiont genotype on symbiont density within
342 polyps, the ability of the symbiont to infect and thrive in the host likely depends on multiple

343 factors (Fig. 3), with potential for variation in the establishment and maintenance of the
344 symbiosis (Davy et al. 2012; Hawkins et al. 2016).

345 Our work highlights that *in vitro* studies of symbiont success under heat stress should not
346 be used as direct predictors of holobiont success. Despite this caveat, the overall thermal
347 tolerance recorded *in vitro* was observed in the polyp experiment, even though performance *in*
348 *vitro* was not directly mirrored *in hospite*. All treatments had relatively high polyp survival at
349 one month, with a more rapid decline in survivorship over the second month (Fig. 4a, b). Polyps
350 showed sustained survivorship over an extended period of heat exposure, with some surviving
351 polyps in all treatments at 56 days post-inoculation in spite of the algal overgrowth. This is a
352 substantially longer exposure to heat stress than has been previously reported for *in hospite*
353 experiments (i.e., Chakravarti et al. 2017: 28 days; Buerger et al. 2020: 7 days). Our results
354 suggest that the observed thermal tolerance exhibited by our symbiont types may have
355 contributed to or enhanced the thermal tolerance of the holobiont, although the long-term value
356 of this has yet to be determined.

357 In this study, the host-symbiont pairs exhibited thermal tolerance. *Breviolum* cultures
358 grown at 30°C did not show enhanced performance at 30°C (as compared to 26°C). This
359 indicates that selection for thermal tolerance of certain genotypes within the colonies likely
360 occurred prior to the isolation of these symbionts. It is possible that thermal tolerance has been
361 selected for in *Breviolum* over much longer time periods than those in this experiment in
362 response to historical thermal events. Studies of both long- and short-term responses to thermal
363 stress show that these organisms can successfully function at elevated temperatures, suggesting
364 that adaptation or acclimatization may ameliorate holobiont responses to thermal stress.
365 Holobionts may have adapted to variation in temperature regimes over thousands of years

366 (Smith-Keune and van Oppen 2006; Howells et al. 2013, 2016; Hume et al. 2015; Kenkel and
367 Matz 2016). For example, corals found in naturally high-temperature environments (e.g.,
368 Persian/Arabian Gulf, warm water pools) show increased thermal tolerance compared to
369 conspecifics from cooler habitats (Howells et al. 2013, 2016; Bay and Palumbi 2014; Hume et al.
370 2015). Rapid adaptation or acclimatization may increase thermal tolerance over shorter time
371 scales, exemplified by reduced levels of bleaching in the years following extreme bleaching
372 events (Maynard et al. 2008; Guest et al. 2012; Pratchett et al. 2013; Logan et al. 2014;
373 McClanahan et al. 2014; Brown et al. 2015; Coles et al. 2018).

374 Ultimately, our results indicate that there exists standing genetic variation within
375 symbiont populations, which in this case, contained genotypes that were thermally tolerant.
376 These symbionts may be vital for the holobiont's survival, although the basis for this thermal
377 tolerance requires further investigation. Various other factors including host-symbiont GxG
378 interactions and alternative selection pressures may influence holobiont success at elevated
379 temperatures (Baird et al. 2009; Chakravarti et al. 2017; Gabay et al. 2019). Additionally,
380 interactions between the entire coral microbiome (including endosymbiotic dinoflagellates,
381 bacteria, viruses and fungi) may be key in determining thermal stress responses of the holobiont
382 (Vega Thurber et al. 2009; Littman et al. 2011; Bourne et al. 2016).

383 **Role of the symbiont and implications for Caribbean reefs**

384 Most previous studies of the capacity for rapid thermal adaptation in symbionts have
385 focused on Pacific strains of Symbiodiniaceae. Our results shed new light on the existence of
386 thermally tolerant Caribbean coral endosymbionts in genera in addition to those thermally
387 tolerant species within the genus *Durusdinium* (LaJeunesse et al. 2014). We provide key
388 evidence that even in the absence of rapid adaptation to elevated temperatures, thermal tolerance

389 may be widespread in some *Breviolum* species. It is likely that broad thermal tolerance is an
390 inherent trait among these genotypes and perhaps among other species within *Breviolum*,
391 particularly those within the B1 (ITS2-type) lineage (Pleistocene (B1) radiation, *sensu*
392 Lajeunesse 2005; Parkinson et al. 2015) such as *B. antillogorgium*. During thermal stress, for
393 example, the octocoral *Briareum asbestinum* lost its dominant *Breviolum* ITS2 type B19
394 symbiont (Poland et al. 2013), which was replaced by a *Breviolum* ITS2 type B1 (Lewis and
395 Coffroth 2004; Poland and Coffroth 2019). Transitioning from a dominant symbiont species with
396 lower thermal tolerance to a more thermally tolerant cryptic species during bleaching events may
397 buffer Caribbean octocorals from increasing temperatures.

398 Coral reefs are in decline worldwide and some regions, such as the Caribbean, are
399 experiencing a phase shift from scleractinian-dominated to octocoral-dominated ecosystems
400 (Ruzicka et al. 2013; Lenz et al. 2015; Lasker et al. 2020). This pattern has been attributed, in
401 part, to high levels of octocoral recruitment (Lasker et al. 2020). However, bleaching in
402 octocorals is rarely observed during mass bleaching events (Lasker et al. 1984; Lasker 2003;
403 Prada et al. 2010) and if it is observed, most species show a high level of resilience (Prada et al.
404 2010). The fact that species within *Breviolum* are the dominant symbionts in Caribbean
405 octocorals (van Oppen et al. 2005; Goulet et al. 2008; Franklin et al. 2012), and that some
406 symbiont species within this genus appear to be thermally tolerant, as shown here, may
407 contribute to this resilience. Additional studies are needed to determine the prevalence of these
408 thermally tolerant symbionts, that tend to exist at background level, and their role in mitigating
409 the effects of increasing temperatures on reef cnidarians. The survival of Caribbean reefs may be
410 reliant on evolutionary rescue (Gomulkiewicz and Holt 1995) perpetrated by selection for these
411 more thermally tolerant symbiont genotypes.

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Conflict of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

Andras JP, Kirk NL, Coffroth MA, Harvell CD (2009) Isolation and characterization of microsatellite loci in Symbiodinium B1/B184, the dinoflagellate symbiont of the Caribbean sea fan coral, *Gorgonia ventalina*. Mol. Ecol. Resour. 9:989–993

Baird AH, Bhagooli R, Ralph PJ, Takahashi S (2009) Coral bleaching: the role of the host. Trends Ecol Evol (Amst) 24:16–20

Baker AC (2003) Flexibility and Specificity in Coral-Algal Symbiosis: Diversity, Ecology, and Biogeography of *Symbiodinium*. Annu. Rev. Ecol. Evol. Syst. 34:661–689

Baums IB, Devlin-Durante MK, LaJeunesse TC (2014) New insights into the dynamics between reef corals and their associated dinoflagellate endosymbionts from population genetic studies. Mol. Ecol. 23:4203–4215

Bayliss SLJ, Scott ZR, Coffroth MA, terHorst CP (2019) Genetic variation in *Breviolum antillogorgium*, a coral reef symbiont, in response to temperature and nutrients. Ecol. Evol. 9:2803–2813

Bay RA, Palumbi SR (2014) Multilocus adaptation associated with heat resistance in reef-building corals. Curr. Biol. 24:2952–2956

Berkelmans R, van Oppen MJH (2006) The role of zooxanthellae in the thermal tolerance of corals: a “nugget of hope” for coral reefs in an era of climate change. Proc. Biol. Sci. 273:2305–2312

Bernhardt JR, Sunday JM, O’Connor MI (2018) Metabolic Theory and the Temperature-Size Rule Explain the Temperature Dependence of Population Carrying Capacity. Am. Nat. 192:687–697

442 Bourne DG, Morrow KM, Webster NS (2016) Insights into the Coral Microbiome: Underpinning
443 the Health and Resilience of Reef Ecosystems. Annu. Rev. Microbiol. 70:317–340

444 Brown BE, Dunne RP, Edwards AJ, Sweet MJ, Phongsuwan N (2015) Decadal environmental
445 'memory' in a reef coral? Mar. Biol. 162:479–483

446 Buerger P, Alvarez-Roa C, Coppin CW, Pearce SL, Chakravarti LJ, Oakeshott JG, Edwards OR,
447 van Oppen MJH (2020) Heat-evolved microalgal symbionts increase coral bleaching
448 tolerance. Sci. Adv. 6:eaba2498

449 Chakravarti LJ, Beltran VH, van Oppen MJH (2017) Rapid thermal adaptation in
450 photosymbionts of reef-building corals. Glob. Chang. Biol. 23:4675–4688

451 Chakravarti LJ, van Oppen MJH (2018) Experimental evolution in coral photosymbionts as a
452 tool to increase thermal tolerance. Front. Mar. Sci. 5:

453 Chi J, Parrow MW, Dunthorn M (2014) Cryptic sex in *Symbiodinium* (Alveolata, Dinoflagellata)
454 is supported by an inventory of meiotic genes. J. Eukaryot. Microbiol. 61:322–327

455 Coffroth MA, Lasker HR, Diamond ME, Bruenn JA, Bermingham E (1992) DNA fingerprints of
456 a gorgonian coral: a method for detecting clonal structure in a vegetative species. Mar. Biol.
457 114:317–325

458 Coles SL, Bahr KD, Rodgers KS, May SL, McGowan AE, Tsang A, Bumgarner J, Han JH
459 (2018) Evidence of acclimatization or adaptation in Hawaiian corals to higher ocean
460 temperatures. PeerJ 6:e5347

461 Correa AMS, McDonald MD, Baker AC (2009) Development of clade-specific *Symbiodinium*
462 primers for quantitative PCR (qPCR) and their application to detecting clade D symbionts
463 in Caribbean corals. Mar. Biol. 156:2403–2411

464 Davy SK, Allemand D, Weis VM (2012) Cell biology of cnidarian-dinoflagellate symbiosis.
465 Microbiol. Mol. Biol. Rev. 76:229–261

466 Díaz-Almeyda EM, Prada C, Ohdera AH, Moran H, Civitello DJ, Iglesias-Prieto R, Carlo TA,
467 LaJeunesse TC, Medina M (2017) Intraspecific and interspecific variation in
468 thermotolerance and photoacclimation in *Symbiodinium* dinoflagellates. Proc. Biol. Sci.
469 284:

470 Eakin CM, Lough JM, Heron SF (2009) Climate variability and change: monitoring data and
471 evidence for increased coral bleaching stress. In: van Oppen M.J.H., Lough J.M. (eds) Coral
472 Bleaching. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 41–67

473 Franklin EC, Stat M, Pochon X, Putnam HM, Gates RD (2012) GeoSymbio: a hybrid, cloud-
474 based web application of global geospatial bioinformatics and ecoinformatics for
475 *Symbiodinium*-host symbioses. Mol. Ecol. Resour. 12:369–373

476 Gabay Y, Parkinson JE, Wilkinson SP, Weis VM, Davy SK (2019) Inter-partner specificity
477 limits the acquisition of thermotolerant symbionts in a model cnidarian-dinoflagellate
478 symbiosis. ISME J. 13:2489–2499

479 Gomulkiewicz R, Holt RD (1995) When does evolution by natural selection prevent extinction?
480 Evolution 49:201

481 Goulet TL (2006) Most corals may not change their symbionts. Mar. Ecol. Prog. Ser. 321:1–7

482 Goulet TL, Simmons C, Goulet D (2008) Worldwide biogeography of *Symbiodinium* in tropical
483 octocorals. Mar. Ecol. Prog. Ser. 355:45–58
484
485 Grégoire V, Schmacka F, Coffroth MA, Karsten U (2017) Photophysiological and thermal
486 tolerance of various genotypes of the coral endosymbiont *Symbiodinium* sp. (Dinophyceae).
487 J. Appl. Phycol. 29:1893–1905
488 Guest JR, Baird AH, Maynard JA, Muttaqin E, Edwards AJ, Campbell SJ, Yewdall K, Affendi
489 YA, Chou LM (2012) Contrasting patterns of coral bleaching susceptibility in 2010 suggest
490 an adaptive response to thermal stress. PLoS ONE 7:e33353
491
492 Guillard RR, Ryther JH (1962) Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt,
493 and *Detonula confervacea* (Cleve) Gran. Can. J. Microbiol. 8:229–239
494 Hawkins TD, Hagemeyer JCG, Warner ME (2016) Temperature moderates the infectiousness of
495 two conspecific *Symbiodinium* strains isolated from the same host population. Environ.
496 Microbiol. 18:5204–5217
497 Heron SF, Maynard JA, van Hooidonk R, Eakin CM (2016) Warming Trends and Bleaching
498 Stress of the World's Coral Reefs 1985-2012. Sci. Rep. 6:38402
499 Hoegh-Guldberg O (1999) Climate change, coral bleaching and the future of the world's coral
500 reefs. Mar. Freshwater Res. 50:839
501 Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Greenfield P, Gomez E, Harvell CD,
502 Sale PF, Edwards AJ, Caldeira K, Knowlton N, Eakin CM, Iglesias-Prieto R, Muthiga N,
503 Bradbury RH, Dubi A, Hatziolos ME (2007) Coral reefs under rapid climate change and
504 ocean acidification. Science 318:1737–1742
505 Hoegh-Guldberg O (2011) Coral reef ecosystems and anthropogenic climate change. Reg.
506 Environ. Change 11:215–227
507 Hoegh-Guldberg O, Poloczanska ES, Skirving W, Dove S (2017) Coral Reef Ecosystems under
508 Climate Change and Ocean Acidification. Front. Mar. Sci. 4:
509 Howells EJ, van Oppen MJH, Willis BL (2009) High genetic differentiation and cross-shelf
510 patterns of genetic diversity among Great Barrier Reef populations of *Symbiodinium*. Coral
511 Reefs 28:215–225
512 Howells EJ, Beltran VH, Larsen NW, Bay LK, Willis BL, van Oppen MJH (2011) Coral thermal
513 tolerance shaped by local adaptation of photosymbionts. Nat. Clim. Chang. 2:116–120
514 Howells EJ, Berkelmans R, van Oppen MJH, Willis BL, Bay LK (2013) Historical thermal
515 regimes define limits to coral acclimatization. Ecology 94:1078–1088
516 Howells EJ, Abrego D, Meyer E, Kirk NL, Burt JA (2016) Host adaptation and unexpected
517 symbiont partners enable reef-building corals to tolerate extreme temperatures. Glob.
518 Chang. Biol. 22:2702–2714
519 Hughes TP, Anderson KD, Connolly SR, Heron SF, Kerry JT, Lough JM, Baird AH, Baum JK,
520 Berumen ML, Bridge TC, Claar DC, Eakin CM, Gilmour JP, Graham NAJ, Harrison H,
521 Hobbs J-PA, Hoey AS, Hoogenboom M, Lowe RJ, McCulloch MT, Wilson SK (2018)

Spatial and temporal patterns of mass bleaching of corals in the Anthropocene. Science 359:80–83

Hume BCC, D'Angelo C, Smith EG, Stevens JR, Burt J, Wiedenmann J (2015) *Symbiodinium thermophilum* sp. nov., a thermotolerant symbiotic alga prevalent in corals of the world's hottest sea, the Persian/Arabian Gulf. Sci. Rep. 5:8562

Jones AM, Berkelmans R, van Oppen MJH, Mieog JC, Sinclair W (2008) A community change in the algal endosymbionts of a scleractinian coral following a natural bleaching event: field evidence of acclimatization. Proc. Biol. Sci. 275:1359–1365

Kahng SE, Benayahu Y, Lasker HR (2011) Sexual reproduction in octocorals. Mar. Ecol. Prog. Ser. 443:265–283

Karim W, Nakaema S, Hidaka M (2015) Temperature effects on the growth rates and photosynthetic activities of *Symbiodinium* cells. J. Mar. Sci. Eng. 3:368–381

Kenkel CD, Matz MV (2016) Gene expression plasticity as a mechanism of coral adaptation to a variable environment. Nat. Ecol. Evol. 1:14

Keshavmurthy S, Hsu C-M, Kuo C-Y, Meng P-J, Wang J-T, Chen CA (2012) Symbiont communities and host genetic structure of the brain coral *Platygyra verweyi*, at the outlet of a nuclear power plant and adjacent areas. Mol. Ecol. 21:4393–4407

Kirk NL, Andras JP, Harvell CD, Santos SR, Coffroth MA (2009) Population structure of *Symbiodinium* sp. associated with the common sea fan, *Gorgonia ventalina*, in the Florida Keys across distance, depth, and time. Mar. Biol. 156:1609–1623

LaJeunesse T (2002) Diversity and community structure of symbiotic dinoflagellates from Caribbean coral reefs. Mar. Biol. 141:387–400

LaJeunesse TC (2005) "Species" radiations of symbiotic dinoflagellates in the Atlantic and Indo-Pacific since the Miocene-Pliocene transition. Mol. Biol. Evol. 22:570–581

LaJeunesse TC, Smith R, Walther M, Pinzón J, Pettay DT, McGinley M, Aschaffenburg M, Medina-Rosas P, Cupul-Magaña AL, Pérez AL, Reyes-Bonilla H, Warner ME (2010) Host-symbiont recombination versus natural selection in the response of coral-dinoflagellate symbioses to environmental disturbance. Proc. Biol. Sci. 277:2925–2934

LaJeunesse TC, Parkinson JE, Reimer JD (2012) A genetics-based description of *Symbiodinium minutum* sp. nov. and *S. psygmophilum* sp. nov. (Dinophyceae), two dinoflagellates symbiotic with cnidaria. J. Phycol. 48:1380–1391

LaJeunesse TC, Wham DC, Pettay DT, Parkinson JE, Keshavmurthy S, Chen CA (2014) Ecologically differentiated stress-tolerant endosymbionts in the dinoflagellate genus*Symbiodinium* (Dinophyceae) Clade D are different species. Phycologia 53:305–319

LaJeunesse T (2020) Zooxanthellae. Curr Biol 30:R1110–R1113

Lasker HR, Peters EC, Coffroth MA (1984) Bleaching of reef coelenterates in the San Blas Islands, Panama. Coral Reefs 3:183–190

Lasker HR (2003) Zooxanthella densities within a Caribbean octocoral during bleaching and non-bleaching years. Coral Reefs 22:23–26

562 Lasker HR, Martínez-Quintana Á, Bramanti L, Edmunds PJ (2020) Resilience of octocoral
563 forests to catastrophic storms. Sci. Rep. 10:4286

564 Lenz EA, Bramanti L, Lasker HR, Edmunds PJ (2015) Long-term variation of octocoral
565 populations in St. John, US Virgin Islands. Coral Reefs 34:1099–1109

566 Lewis CL, Coffroth MA (2004) The acquisition of exogenous algal symbionts by an octocoral
567 after bleaching. Science 304:1490–1492

568 Littman R, Willis BL, Bourne DG (2011) Metagenomic analysis of the coral holobiont during a
569 natural bleaching event on the Great Barrier Reef. Environ. Microbiol. Rep. 3:651–660

570 Logan CA, Dunne JP, Eakin CM, Donner SD (2014) Incorporating adaptive responses into
571 future projections of coral bleaching. Glob. Chang. Biol. 20:125–139

572 Maynard JA, Anthony KRN, Marshall PA, Masiri I (2008) Major bleaching events can lead to
573 increased thermal tolerance in corals. Mar. Biol. 155:173–182

574 McClanahan TR, Ateweberhan M, Darling ES, Graham NAJ, Muthiga NA (2014) Biogeography
575 and change among regional coral communities across the Western Indian Ocean. PLoS
576 ONE 9:e93385

577 Mieog JC, van Oppen MJH, Cantin NE, Stam WT, Olsen JL (2007) Real-time PCR reveals a
578 high incidence of *Symbiodinium* clade D at low levels in four scleractinian corals across the
579 Great Barrier Reef: implications for symbiont shuffling. Coral Reefs 26:449–457

580 Muscatine L, Cernichiari E (1969) Assimilation of photosynthetic products of zooxanthellae by a
581 reef coral. Biol. Bull. 137:506–523

582 Muscatine L, Porter JW (1977) Reef Corals: Mutualistic Symbioses Adapted to Nutrient-Poor
583 Environments. Bioscience 27:454–460

584 Newkirk CR, Frazer TK, Martindale MQ, Schnitzler CE (2020) Adaptation to bleaching: are
585 thermotolerant symbiodiniaceae strains more successful than other strains under elevated
586 temperatures in a model symbiotic cnidarian? Front. Microbiol. 11:822

587 Oliver JK, Berkelmans R, Eakin CM (2018) Coral bleaching in space and time. Coral Bleaching.
588 Springer, pp 27–49

589 Parkinson JE, Coffroth MA, LaJeunesse TC (2015) New species of Clade B *Symbiodinium*
590 (Dinophyceae) from the greater Caribbean belong to different functional guilds:
591 *S. aenigmaticum* sp. nov., *S. antillogorgium* sp. nov., *S. endomadracis* sp. nov., and
592 *S. pseudominutum* sp. nov. J. Phycol. 51:850–858

593 Pettay DT, Lajeunesse TC (2007) Microsatellites from clade B *Symbiodinium* spp. specialized
594 for Caribbean corals in the genus *Madracis*. Mol. Ecol. Notes 7:1271–1274

595 Petzoldt T (2019) growthrates. Available at: https://github.com/tpetzoldt/growthrates

596 Poland DM, Mansfield JM, Hannes AR, Lewis CLF, Shearer TL, Connelly SJ, Kirk NL,
597 Coffroth MA (2013) Variation in Symbiodinium communities in juvenile Briareum
598 asbestinum (Cnidaria: Octocorallia) over temporal and spatial scales. Mar. Ecol. Prog. Ser.
599 476:23–37

600 Poland DM, Coffroth MA (2017) Trans-generational specificity within a cnidarian–algal
601 symbiosis. Coral Reefs 36:119–129

602 Poland DM, Coffroth MA (2019) Host growth and survivorship varies with endosymbiotic algal
603 partner in developing cnidarians. Mar. Ecol. Prog. Ser. 612:87–100

604 Prada C, Weil E, Yoshioka PM (2010) Octocoral bleaching during unusual thermal stress. Coral
605 Reefs 29:41–45

606 Pratchett MS, McCowan D, Maynard JA, Heron SF (2013) Changes in bleaching susceptibility
607 among corals subject to ocean warming and recurrent bleaching in Moorea, French
608 Polynesia. PLoS ONE 8:e70443

609 Quigley K.M., Baker A.C., Coffroth M.A., Willis B.L., van Oppen M.J.H. (2018) Bleaching
610 Resistance and the Role of Algal Endosymbionts. Ch. 6 pp. 111-151. In: Coral Bleaching:
611 Patterns, Processes, Causes and Consequences eds; J. Lough and M. van Oppen. Springer
612 Ecological Series. Cham, Switzerland

613 R Core Team (2020). R: A language and environment for statistical computing. R Foundation for
614 Statistical Computing, Vienna, Astria. URL https://www.R-project.org/.

615 Ruzicka RR, Colella MA, Porter JW, Morrison JM, Kidney JA, Brinkhuis V, Lunz KS,
616 Macaulay KA, Bartlett LA, Meyers MK, Colee J (2013) Temporal changes in benthic
617 assemblages on Florida Keys reefs 11 years after the 1997/1998 El Niño. Mar. Ecol. Prog.
618 Ser. 489:125–141

619 Santos SR, Taylor DJ, Coffroth MA (2001) Genetic comparisons of freshly isolated versus
620 cultured symbiotic dinoflagellates: implications for extrapolating to the intact symbiosis. J.
621 Phycol. 37:900–912

622 Santos SR, Guti□rrez-Rodr□guez C, Lasker HR, Coffroth MA (2003) *Symbiodinium* sp.
623 associations in the gorgonian *Pseudopterogorgia elisabethae* in the Bahamas: high levels of
624 genetic variability and population structure in symbiotic dinoflagellates. Mar. Biol.
625 143:111–120

626 Santos SR, Shearer TL, Hannes AR, Coffroth MA (2004) Fine-scale diversity and specificity in
627 the most prevalent lineage of symbiotic dinoflagellates (*Symbiodinium*, Dinophyceae) of the
628 Caribbean. Mol. Ecol. 13:459–469

629 Savage VM, Gilloly JF, Brown JH, Charnov EL (2004) Effects of body size and temperature on
630 population growth. Am. Nat. 163:429–441

631 Silverstein RN, Correa AMS, Baker AC (2012) Specificity is rarely absolute in coral-algal
632 symbiosis: implications for coral response to climate change. Proc. Biol. Sci. 279:2609–
633 2618

634 Silverstein RN, Cunning R, Baker AC (2015) Change in algal symbiont communities after
635 bleaching, not prior heat exposure, increases heat tolerance of reef corals. Glob. Chang.
636 Biol. 21:236–249

637 Smith-Keune C, van Oppen M (2006) Genetic structure of a reef-building coral from thermally
638 distinct environments on the Great Barrier Reef. Coral Reefs 25:493–502

639 Thornhill DJ, Howells EJ, Wham DC, Steury TD, Santos SR (2017) Population genetics of reef
640 coral endosymbionts (*Symbiodinium*, Dinophyceae). Mol. Ecol. 26:2640–2659

641 Torda G, Donelson JM, Aranda M, Barshis DJ, Bay L, Berumen ML, Bourne DG, Cantin N,
642 Foret S, Matz M, Miller DJ, Moya A, Putnam HM, Ravasi T, van Oppen MJH, Thurber RV,
643 Vidal-Dupiol J, Voolstra CR, Watson S-A, Whitelaw E, Munday PL (2017) Rapid adaptive
644 responses to climate change in corals. Nat. Clim. Chang. 7:627–636

645 Tukey JW (1949) Comparing individual means in the analysis of variance. Biometrics 5:99–
646 114.

647 van Hooidonk R, Maynard J, Tamelander J, Gove J, Ahmadia G, Raymundo L, Williams G,
648 Heron SF, Planes S (2016) Local-scale projections of coral reef futures and implications of
649 the Paris Agreement. Sci. Rep. 6:39666

650 van Oppen MJH, Mieog JC, Sánchez CA, Fabricius KE (2005) Diversity of algal endosymbionts
651 (zooxanthellae) in octocorals: the roles of geography and host relationships. Mol. Ecol.
652 14:2403–2417

653 van Oppen MJH, Souter P, Howells EJ, Heyward A, Berkelmans R (2011) Novel genetic
654 diversity through somatic mutations: fuel for adaptation of reef corals? Diversity (Basel)
655 3:405–423

656 Vega Thurber R, Willner-Hall D, Rodriguez-Mueller B, Desnues C, Edwards RA, Angly F,
657 Dinsdale E, Kelly L, Rohwer F (2009) Metagenomic analysis of stressed coral holobionts.
658 Environ. Microbiol. 11:2148–2163

659 Wilkinson SP, Fisher PL, van Oppen MJH, Davy SK (2015) Intra-genomic variation in
660 symbiotic dinoflagellates: recent divergence or recombination between lineages? BMC
661 Evol. Biol. 15:325

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Tables

Table 1. Five genotypes of *Breviolum antillogorgium* selected for *in vitro* and *in hospite* experiments. Letter color refers to selection temperature, blue indicating cultures maintained at 26°C and red indicating cultures maintained at 30°C two years prior to the start of the experiment. Cultures were isolated from colonies collected at Elbow Reef, Florida Keys.

Culture	Genotype	Selection Tempt	Source Colony	Reef	Depth	Location	Accession No.
16-0590F	G-1	26 C	7	Elbow	18 m	N 25 07.925 W 80 15.717	XXX
16-0875	G-2	26 C	5	Elbow	11 m	N 25 07.956 W 80 15.810	XXX
16-1631	G-3	26 C	8	Elbow	18 m	N 25 07.925 W 80 15.717	XXX
16-0587	G-4	30 C	8	Elbow	18 m	N 25 07.925 W 80 15.717	XXX
16-0763	G-5	30 C	8	Elbow	18 m	N 25 07.925 W 80 15.717	XXX

Table 2. Experimental set up for the *in hospite* study. Letter color refers to selection temperature, blue indicating cultures reared at 26 C and red indicating cultures reared at 30 C two years prior to the start of the experiment.

Treatment	Mean # Polyps/container	# polyps/treatment	Containers/ Treatment
G-1 @ 26	13	160	12
G-2 @ 26	13	159	12
G-3 @ 26	13	163	12
G-4 @ 26	13	166	12
G-5 @ 26	14	169	12
G-1 @ 30	8	55	8
G-2 @ 30	8	67	8
G-3 @ 30	8	58	8
G-4 @ 30	8	56	8
G-5 @ 30	8	58	8

710 **Table 3.** P-values for significant pairwise comparisons from Tukey's HSD post-hoc tests of

711 symbiont cell densities in polyps (*in hospite* experiment). No other pairwise comparisons were

712 significant.

Genotype at Temperature	P-values
G1 at 30°C > G3 at 30°C	0.0039
G3 at 26° C >G5 at 26°C	0.0229
G1 at 30°C > G2 at 26°C	0.031
G1 at 30°C >G4 at 30°C	0.045
G1 at 30°C > G5 at 26°C	0.003
G4 at 26°C > G3 at 30°C	0.039

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Figures

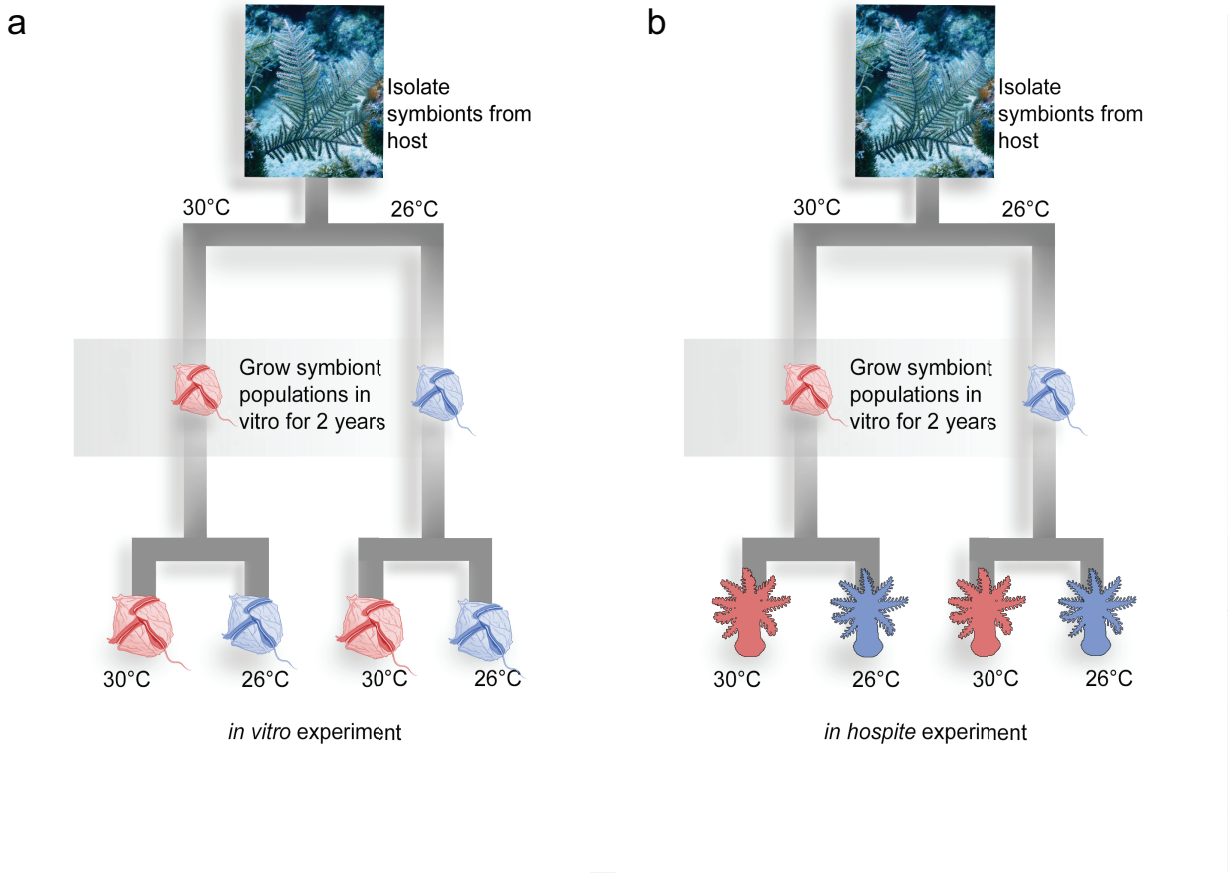

Figure 1. Experimental design for a.) *in vitro* growth experiment and b.) *in hospite* experiment. Figure colors refer to temperature treatment (blue, maintained at 26°C; red, maintained at 30°C), with tip figures colored based on treatment (not historical temperature).

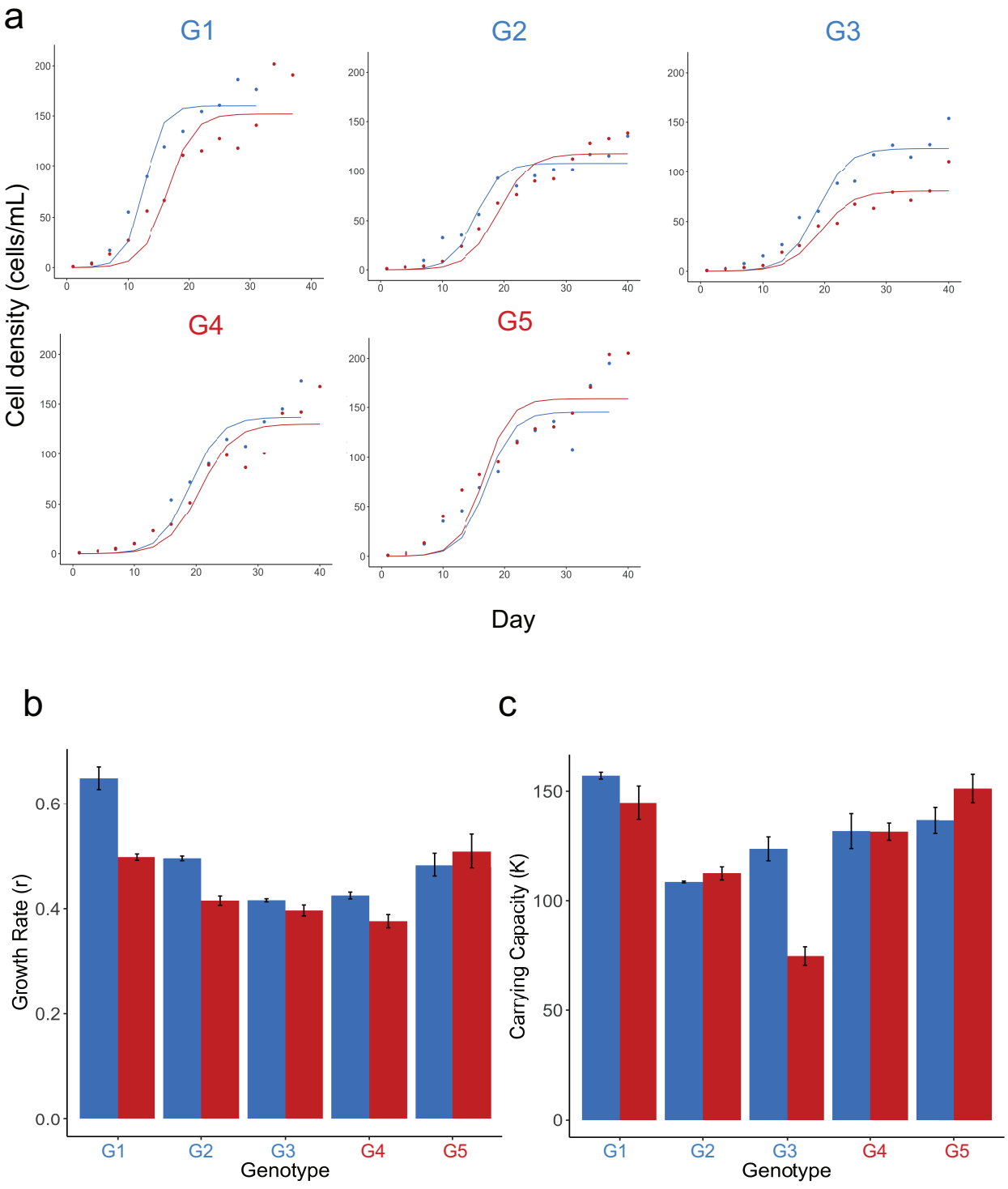

Figure 2. a.) Mean logistic growth fits to *in vitro* cell densities of each genotype of *Breviolum antillogorgium* over time. Blue represents growth at 26°C and red at 30°C. b.) Estimated mean (+/- SE) maximum symbiont population growth rates (r) and C) estimated mean (+/- SE)

735 carrying capacities (K). Genotype colors refer to the historical temperature at which they were
736 grown, blue indicating cultures maintained at 26°C and red indicating cultures maintained at
737 30°C two years prior to the start of the experiment. Bars represent medians, boxes the
738 interquartile range, and whiskers the range of the data.
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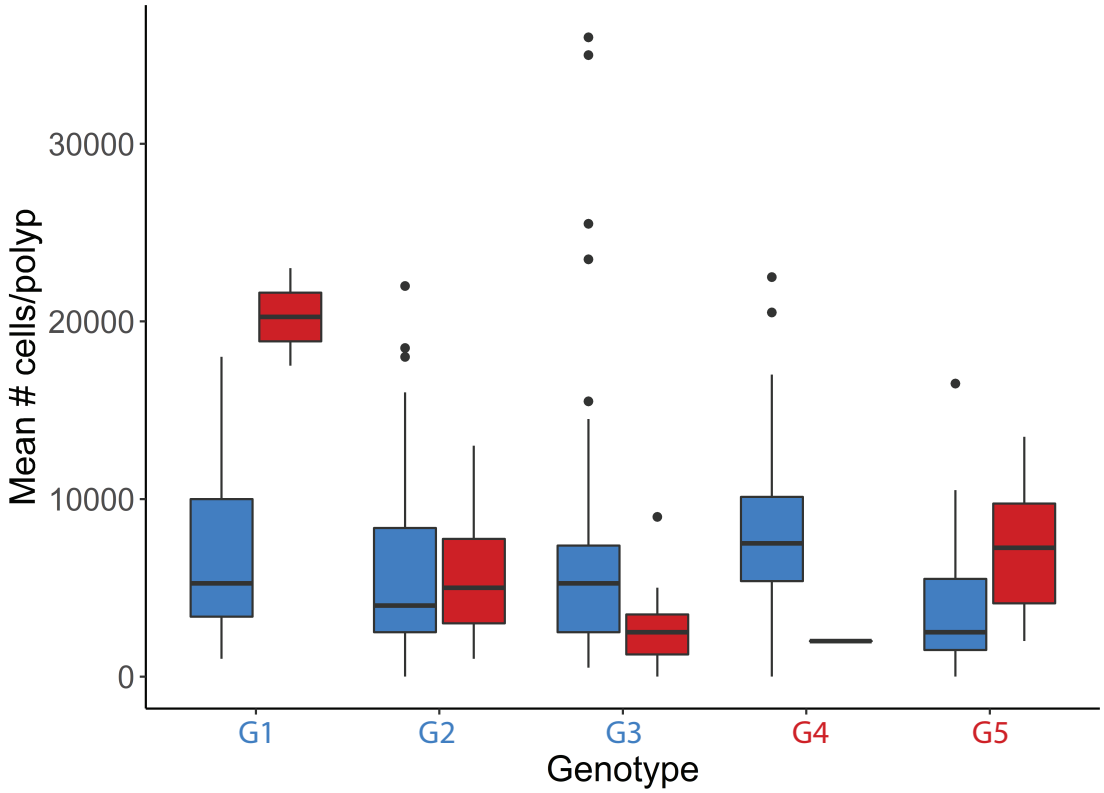

Figure 3. Symbiont densities within *Antillogorgia bipinnata* polyps inoculated with five symbiont genotypes at 26°C and 30°C. Statistically significant differences for these measures in Table 3. Genotype color refers to historical temperature, blue indicating cultures maintained at 26°C and red indicating cultures maintained at 30°C two years prior to the start of the experiment. Boxes represent interquartile range and whiskers the range of the data. Points indicate outliers.

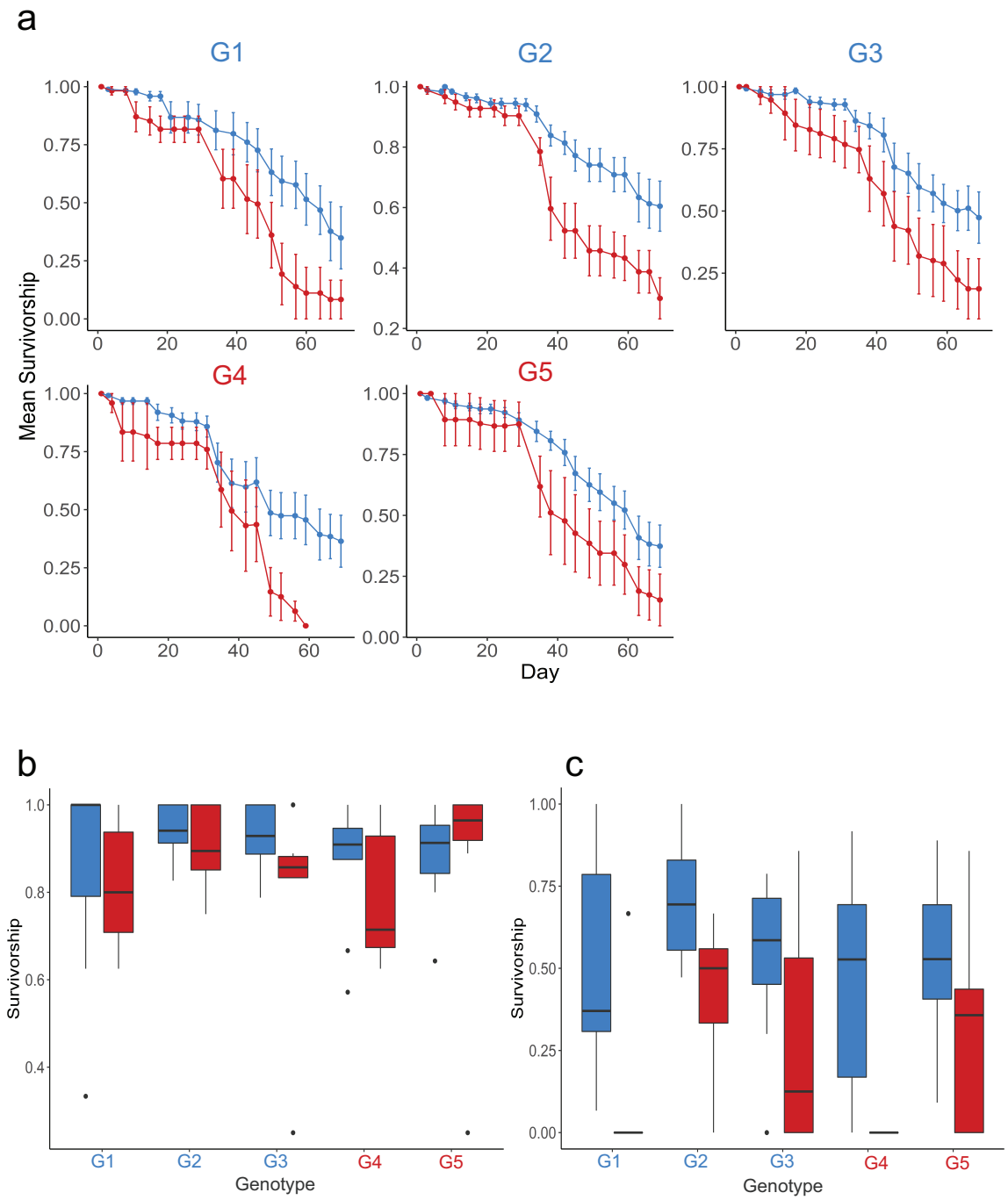

Figure 4. a.) Mean polyp survivorship over time for *A. bipinnata* polyps inoculated with five symbiont genotypes at 26°C and 30°C. Error bars are ±SE. Blue lines refer to treatments at 26°C and red lines refer to treatments at 30°C. Differences in average survivorship at the end of the b.)

760 first month and c.) second month. Genotype color refers to historical temperature, blue indicating
761 cultures maintained at 26°C and red indicating cultures maintained at 30°C two years prior to the
762 start of the experiment. Boxes represent interquartile range and whiskers the range of the data.
763 Points indicate outliers.