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Temperature and *Symbiodinium* physiology affect the establishment and development of symbiosis in corals

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ABSTRACT: Symbiotic associations are ubiquitous in nature. In fact, all eukaryotic species harbour microbial symbionts that are essential for their health. Often overlooked, symbiosis is an important factor when predicting how organisms might respond to climate change. Some associations are so tight-knit that rapid changes in the environment can lead to extinction of one or both partners. Alternatively, the ability to switch to more stress-tolerant partners can allow for rapid adjustment to environmental change, such as increases in host range size. Here, we outline a mechanism by which symbiotic species that acquire their symbionts anew each generation might adapt to global warming via transgenerational, environmentally mediated changes in host–symbiont partnerships. At temperatures approximating climate change conditions at the end of the century, the larvae of 2 common scleractinian corals established symbiosis with a novel and more thermo-tolerant symbiont. Conversely, the establishment of symbiosis with heat-sensitive symbionts was greatly reduced. Transgenerational change in symbionts is a mechanism by which organisms that engage in flexible mutualistic relationships can rapidly adjust to a changing climate.

KEY WORDS: Acclimatisation · *Acropora millepora* · *Acropora monticulosa* · Coral reefs · Climate change · Larval ecology · Symbiosis · *Symbiodinium*

INTRODUCTION

Symbiosis can be an opportunity or a challenge for species when confronted with rapid environmental change. The ability to associate with multiple symbiotic partners can confer plasticity that accelerates adaptation (Buddemeier & Fautin 1993, Daskin & Alford 2012). For example, elevated temperatures select for thermally tolerant genotypes of the obligate mutualist bacteria *Wolbachia* sp., thereby conferring heat resistance to its host, the wasp *Trichogramma cordubensis* (Pintureau et al. 1999). Additionally, the ability to associate with multiple types of bacterial endosymbionts allows an aphid host to avoid parasitism under heat stress (Guay et al. 2009). In contrast, if the association between the host and symbiont is highly specific, changes in abiotic factors can lead to large-scale mortality. For example, the association between adult corals and their symbiotic algae, *Symbiodinium* is highly sensitive to thermal stress, which leads to large-scale bleaching, often followed by mortality during sea temperature anomalies (Hughes et al. 2003, Baird et al. 2009a). Unless the coral–*Symbiodinium* holobiont can acclimatise or adapt to elevations in temperature, climate change threatens the persistence of these ecosystems (Baker et al. 2008, Spalding & Brown 2015).

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One proposed mechanism to cope with such change is for the coral host to shift to more heat-tolerant *Symbiodinium* partners (Buddemeier & Fautin 1993, Glynn et al. 2001, Baker et al. 2004, Berkelmans & van Oppen 2006) because the physiology of the symbiont alters that of the holobiont (the coral–*Symbiodinium* unit). Yet, while 6 *Symbiodinium* clades (A–D, F and G) containing multiple types occur in corals globally (Baker 2003), most coral individuals are dominated by 1 symbiont type (Goulet 2006, Lee et al. 2016) or genotype (Baums et al. 2014). In individuals that host more than 1 symbiont, changes in the relative abundance of symbionts in their tissue (i.e. shuffling) can occur in response to disturbance (Baker et al. 2004, Jones et al. 2008), but there is little evidence to suggest that adult corals can take up and retain novel symbionts from the environment (i.e. switching) (Coffroth et al. 2010). Indeed, much recent research suggests that adult symbioses are stable, even when placed under stress (Sampayo et al. 2016). Nonetheless, coral populations contain individuals that host different symbiont types. This generally occurs when the individuals are from different environmental settings such as different latitudes or depths (Rodriguez-Lanetty et al. 2001, Toller et al. 2001, LaJeunesse et al. 2004, 2010) For example, *Plesiastrea versipora* hosts clade B in temperate regions of south-eastern Australia, while in the subtropical and tropical regions this species associates with clade C (Rodriguez-Lanetty et al. 2001). This indicates that symbiont types are not necessarily species-specific, and suggests that the environment plays a strong role during the establishment and early development of symbiosis.

Approximately 85% of zooxanthellate coral species must establish symbiosis anew in each generation (as larvae or juveniles) from environmental sources, a process known as horizontal transmission (Baird et al. 2009b). During these early life stages, corals are far more flexible in their symbiotic associations than as adults (Cumbo et al. 2013). Importantly, each time a coral breeds, the new generation has an opportunity to acquire novel *Symbiodinium* types more suited to potentially different environments they encounter (LaJeunesse et al. 2004, Baird et al. 2007). We hypothesized that the prevailing environment and the physiology of *Symbiodinium* living outside a host control the establishment of symbiosis and the proliferation of symbionts within the host. We tested this theory by exposing 2 different species of coral larvae to a range of seawater temperatures encompassing present-day and future projected conditions. We offered the larvae 3 *Symbiodinium* types with differing thermal tolerances, and the establishment and early development of symbiosis were determined.

MATERIALS AND METHODS

Coral larvae of 2 common species, *Acropora millepora* and *A. monticulosa*, were used in these experiments. Larvae of these coral species do not initially contain algal symbionts but acquire them during early life stages, making this an ideal system to determine if environment and the physiology of free-living *Symbiodinium* are controlling the establishment of symbiosis. To determine whether corals could acquire any novel *Symbiodinium* types as temperature increases, both *Symbiodinium* that are known to occur in symbiosis or are solely free-living were used.

Thermal tolerance of the *Symbiodinium* cultures

Symbiodinium culture HA3-5 was purchased from the Marine Biotechnology Institute Culture Collection and was a free-living *Symbiodinium* isolated from sediment. Culture CS-159 was purchased from the Australia Commonwealth Scientific and Industrial Research Organisation and was initially isolated from the giant clam *Tridacna maxima*. Culture AT-MI1 was initially isolated from *A. tenuis* collected at Magnetic Island, Queensland, Australia (19° 10′ 6″ S, 146° 50′ 60″ E) in 2005 (collected and isolated by V. R. Cumbo & C. Marquis). Each culture was selected because it represents different phases of *Symbiodinium*, namely a free-living strain (HA3-5), a non-coral-symbiont strain (CS-159) and a coral-symbiont strain (AT-MI1). Cultures were maintained in f/2 medium at 26°C with a light:dark cycle of 12:12 h at a light intensity of approximately 115 µmol photons m^{-2} s^{-1}. *Symbiodinium* identity was assessed by Sanger sequencing of the nuclear rDNA ITS1 region, and cultures were found to be identical to type A (sensu Baillie et al. 2000, GenBank Accession no. AF184948), type A3 (sensu Baillie et al. 2000, AF195143) and type C1 (sensu van Oppen et al. 2001, AF380551), respectively.

The relative thermal tolerance of the 3 *Symbiodinium* types was determined *in vitro* by measuring their maximum quantum yield of photosystem II (F_v/F_m) using pulse amplitude modulated fluorometry (Imaging-PAM). *Symbiodinium* cultures were added to black 24-well Krystal microplates (Povair-Science) at densities of ~6×10^5 cells ml^{-1}, and there were 5 replicates of 2 ml $culture^{-1}$. Their F_v/F_m values

prior to heating were measured (heating day −2), and again after the acclimation period (heating day −1). Cultures were placed in water baths that were maintained at the control temperature (26°C) using a temperature-controlled room, and at 28, 31 and 34°C using aquarium heaters (TH1 thermoregulator, Raytek) and chillers (Unistar-85). Temperature loggers (TinyTag) continuously recorded the temperature in each water bath, and the average temperature for each treatment (±SE) was 25.9 ± 0.003 (n = 2387), 27.9 ± 0.002 (n = 2391), 30.8 ± 0.001 (n = 2385) and 34.3 ± 0.003°C (n = 2391). Illumination was provided with fluorescent bulbs on a 12:12 h light:dark cycle of 115 µmol photons m^{-2} s^{-1}. Photochemical yield (F_v/F_m) measurements were taken on dark-adapted cultures prior to illumination each day over an 8 d period, which included the acclimation day.

Larval collection

Four *A. millepora* colonies were collected on 10 November 2006 from Pioneer Bay, Orpheus Island, in the central Great Barrier Reef (GBR), Australia (18° 36′ 15″ S, 146° 29′ 02″ S) and placed in outdoor aquaria with running filtered seawater (FSW). Colonies were isolated before dusk, and all colonies spawned at approximately 20:00 h. Larvae were cultured following Graham et al. (2008) and maintained in 0.2 µm FSW. Larvae were transported to James Cook University on 13 November 2006 where water changes were performed daily until the beginning of the experiment on 16 November 2006. In 2007, 6 *A. monticulosa* colonies were collected from the southwest side of Akajima Island, Japan (26° 10′ 54″ N, 127° 16′ 19″ E) and placed in tanks with running seawater at the Akajima Marine Science Laboratory. Spawning occurred on 4 August 2007 between 23:00 and 23:30 h. Eggs and sperm were collected, fertilized and then reared in filtered seawater (0.2 µm). Two-day-old, swimming larvae were transported to Sesoko Station, Tropical Biosphere Research Center, University of the Ryukyus, Okinawa, Japan, and maintained as described above.

Symbiont acquisition experiments

Symbiodinium cultures were maintained at James Cook University under the conditions outlined above. In 2007, an aliquot of each culture was transported to Japan and maintained in f/2 medium at 26°C with a light:dark cycle of 12:12 h at a light intensity of approximately 100 µmol photons m^{-2} s^{-1} for 1 mo prior to the second symbiont acquisition experiment. Symbiont acquisition experiments were set up 6 d post spawning, when the larval oral pore and coelenteron were developed, which enables symbiont uptake in these species (Harii et al. 2009).

A. millepora larvae were exposed to each *Symbiodinium* culture while being maintained at 5 temperatures (25, 26, 28, 30 and 32°C) that approximated the daily summer sea surface temperature (SST) range (25–28°C) at Orpheus Island plus 2 high temperature treatments (30 and 32°C) to simulate projected increases in SSTs by 2050–2100 under scenario RCP8.5 (IPCC 2013). Since high irradiance and high temperature can increase photoinhibition in *Symbiodinium*, which could affect establishment and development of symbiosis in the larvae, a high and low light level treatment were included. Larvae of *A. monticulosa* were exposed to each *Symbiodinium* type at 3 different temperatures (25, 28 and 31°C). Temperatures were chosen to cover the daily summer SST range at Akajima Island (25–28°C) (Nadaoka et al. 2001) plus a high temperature treatment (31°C) to simulate projected increases in SST. Only 1 light level was used in this second experiment because the main patterns of establishment and development of symbiosis were not strongly affected by light in the first experiment.

Forty *A. millepora* larvae were placed in 120 ml glass jars containing 0.2 micron-FSW to which *Symbiodinium* were added at densities of 1×10^4 cells ml^{-1}. *Symbiodinium* densities were similar to numbers detected in sediment around coral reefs (Littman et al. 2008). There were 3 jars per *Symbiodinium* type, temperature and light treatment plus 3 jars per temperature and light that contained only larvae and acted as negative controls (i.e. no symbionts added). Jars were placed in water baths at the specified temperatures, which were maintained using heaters (TH1 thermoregulator, Raytek) or chillers (Unistar-85). Temperature loggers (TinyTag) continuously recorded the temperature in each water bath, and the average temperature (±SE) in each bath was 25.3 ± 0.01 (n = 1819), 26.3 ± 0.01 (n = 1885), 28.0 ± 0.003 (n = 1851), 30.0 ± 0.004 (n = 1885) and 32.0 ± 0.005°C (n = 1885). Metal halide lamps created the high light intensity treatment (mean ± SE of 424 ± 6 µmol photons m^{-2} s^{-1}, n = 60), while fluorescent lights generated the low light intensity (39 ± 2 µmol photons m^{-2} s^{-1}, n = 55). Lights were on a 12:12 h light:dark cycle. Ten larvae were sub-sampled from each jar on Days 3 and 6.

Twenty *A. monticulosa* larvae were added to 40 ml jars containing *Symbiodinium* at densities of 1 ×

10^4 cells ml^{-1} (3 jars for each *Symbiodinium* type and temperature plus 3 negative controls per temperature). Light was kept at a constant level of 86 ± 2 µmol photons m^{-2} s^{-1} (±SE, n = 36) using fluorescent light on a 12:12 h light:dark cycle. Temperatures were maintained as above, and were monitored using temperature loggers (HOBO). The average temperature in each bath was 24.9 ± 0.01 (n = 928), 28.01 ± 0.01 (n = 924) and 31.2 ± 0.001°C (n = 924). Eight larvae were sub-sampled from each jar on Days 3 and 6.

To calculate the proportion of larvae establishing symbiosis, the subsampled larvae were inspected after 3 d of exposure to each treatment. Symbiosis was established when the *Symbiodinium* were in the endoderm of the larvae, and this was determined by inspecting larvae under a fluorescent microscope to visualize the chlorophyll fluorescence of the symbionts (Cumbo et al. 2013). To determine if the symbiosis had successfully developed, on Day 6 newly subsampled larvae were inspected under a fluorescent microscope and the number of symbiont cells was counted. Successful development of symbiosis was a result of 2 processes that could not be disentangled, i.e. uptake of *Symbiodinium* cells from the experimental environment and the *in hospite* proliferation of cells.

Statistical analysis

Repeated measures ANOVA tested for differences in the mean photochemical efficiency of photosystem II (F_v/F_m) over time (7 d) between temperatures (25, 28, 31 and 34°C) for each *Symbiodinium* type (A, A3, C1). The initial F_v/F_m (heating day −2, Fig. 1) and acclimation period (heating day −1, Fig. 1) were not included in the analysis. Tukey's post hoc analysis was used to test for differences between temperatures. Differences in the mean proportion of larvae establishing symbiosis among temperatures on Day 3 were determined using 2-way ANOVAs for *A. millepora* and 1-way ANOVAs for *A. monticulosa*. Each physiologically different *Symbiodinium* type was tested independently. Factors in the 2-way ANOVAs were temperature (fixed, 5 levels: 25, 26, 28, 30 and 32°C), and light (fixed, 2 levels: high and low). Temperature (3 levels: 25, 28, 31°C) was the factor in the 1-way ANOVAs. Tukey's post hoc tests were performed to determine the differences between significant temperatures. Differences in the mean *Symbiodinium* densities within the *Symbiodinium*-containing larvae were assessed using 2-way nested ANOVAs for *A. millepora* and 1-way nested ANOVAs for *A. monticulosa*. Factors in the 2-way ANOVAs were temperature and light, with jar nested within temperature × light. Factors within the 1-way ANOVAs were temperature, with jar nested within temperature. The statistical assumptions of normality and homoscedasticity required for ANOVA were tested through graphical analyses of residuals; *Symbiodinium* densities within *A. millepora*

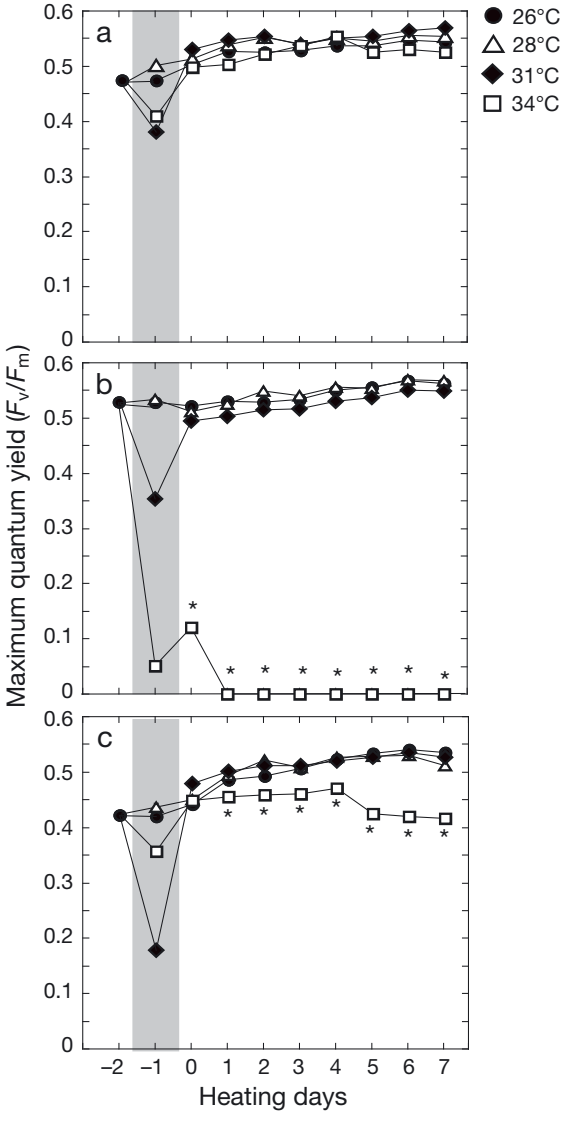

Fig. 1. Thermal sensitivity of *Symbiodinium* types. Maximum quantum yield of photosystem II (F_v/F_m; mean ± SE, n = 5) of *Symbiodinium* types (a) A, (b) A3 and (c) C1 at 26, 28, 31 and 34°C over an 8 d exposure period. Before heating, all cultures were held at 26°C (heating day −2), after which there was a 24 h acclimation period (shaded values). SE too small to be visible. Significant differences between 34°C and the other temperatures on heating days are indicated with asterisks (Tukey post hoc tests; $p < 0.05$)

larvae were square root transformed and *Symbiodinium* densities within *A. monticulosa* larvae were fourth root transformed to meet these assumptions. All analyses were carried out using the statistical program SPSS (version 20.0).

RESULTS

Thermal tolerance of *Symbiodinium* cultures

Thermal tolerance differed markedly among the 3 *Symbiodinium* cultures. Photochemical yield values (F_v/F_m) for *Symbiodinium* type A remained relatively unchanged at temperatures ranging from 26–34°C over the 8 d exposure period (Fig. 1a), indicating no loss of photosynthetic function during extended exposure to ambient and elevated temperatures. In contrast, F_v/F_m in *Symbiodinium* types A3 and C1 declined with increasing exposure to the highest temperature (34°C), while remaining relatively stable at all other temperatures. In type A3, F_v/F_m dropped from 0.53 to 0 after 2 d of exposure to 34°C, indicating a complete loss of photosynthetic function (Fig. 1b). In type C1, F_v/F_m was ~20% lower after 8 d of exposure to 34°C compared to 25–31°C, demonstrating that this type was also sensitive to the high temperature but to a lesser extent than type A3 (Fig. 1c). Therefore, *Symbiodinium* type A was ranked as heat tolerant, while types A3 and C1 were ranked as heat sensitive relative to type A.

Symbiont acquisition at current and future temperatures

Coral larvae successfully acquired all 3 *Symbiodinium* types, but the proportion of larvae establishing symbiosis was strongly affected by temperature and the thermal tolerance of the symbiont type. Establishment of symbiosis with the heat-tolerant *Symbiodinium* A by *Acropora millepora* was high (90–100%) at temperatures between 26 and 32°C, while significantly fewer larvae acquired this symbiont at 25°C ($F_{4,18} = 3.679$, $p = 0.023$, Tukey's < 0.05, Fig. 2a). These establishment patterns were the same under high and low light. In contrast, establishment rates with the heat-sensitive types C1 and A3 were significantly lower at the highest temperature of 32°C (type C1: $F_{4,20} = 40.334$, $p < 0.001$; type A3: $F_{4,20} = 699.727$, $p < 0.001$). Irrespective of the light condition, between 67 and 100% of *A. millepora* larvae acquired type C1 when exposed to temperatures of 25–30°C, while only 20–26% of larvae acquired this type at 32°C (Fig. 2b, Tukey's test $p < 0.05$). Conversely to the uptake patterns of types A and C1, which were unaffected by

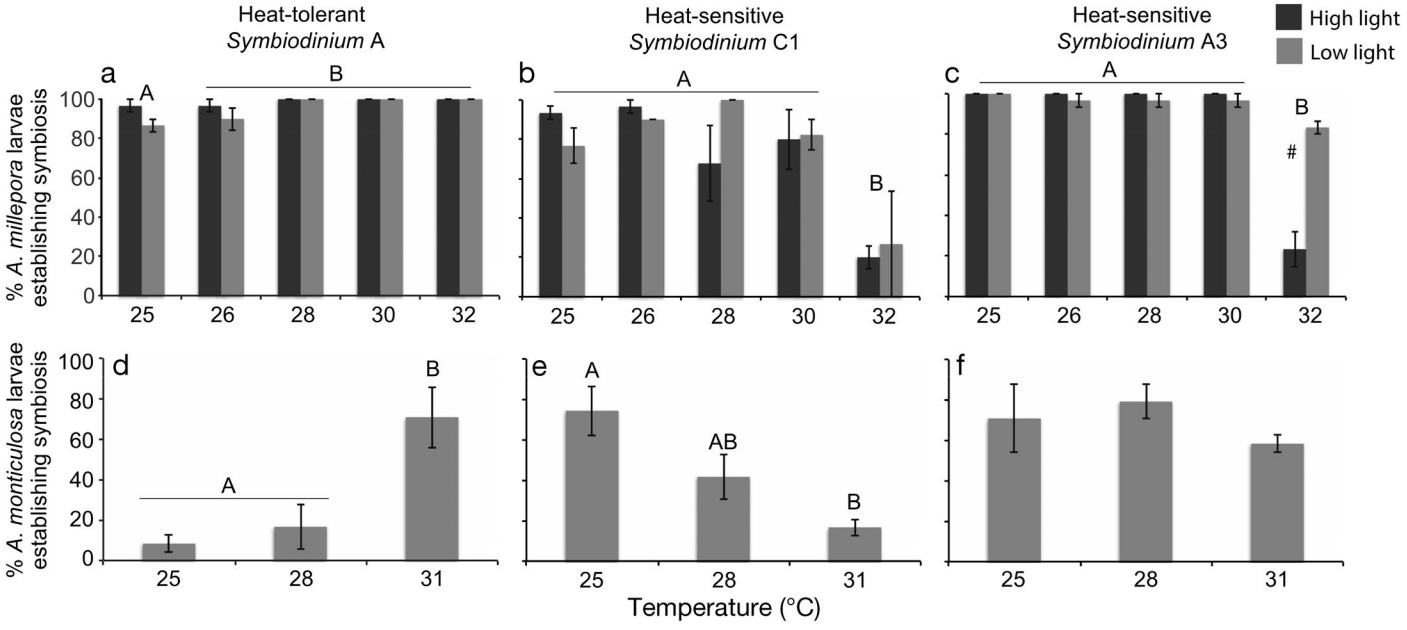

Fig. 2. Percentage (mean ± SE) of coral larvae establishing symbiosis. *Acropora millepora* larvae were exposed to 2 light levels and temperatures ranging from 25–32°C and were presented with *Symbiodinium* (a) heat-tolerant type A, (b) heat-sensitive type C1 and (c) heat-sensitive type A3 for 3 d. (d–f) Similarly, *A. monticulosa* were exposed to 25, 28 and 31°C and given the same *Symbiodinium* types for 3 d. A significant interaction between temperature and light is indicated with a hash (#). Significant differences (Tukey post hoc test; $p < 0.05$) among temperatures are denoted by letters above bars

light, temperature and light interactively affected uptake of the heat-sensitive *Symbiodinium* A3 in *A. millepora* ($F_{4,20}$ = 32.045, p < 0.001). Between 97 and 100% of larvae established symbiosis with this type at temperatures between 25 and 30°C, while at 32°C, 83.3 ± 3.3% (mean ± SE) of larvae acquired type A3 under low light, and only 23.3 ± 8.8% of larvae acquired it under high light (Fig. 2c). Some similar patterns of symbiont acquisition emerged in *A. monticulosa* larvae. The proportion of *A. monticulosa* larvae infected with the heat-tolerant type A was significantly higher at 31°C ($F_{3,6}$ = 14.714, p = 0.004), with 63% and 54% more larvae being infected when compared to 25 and 28°C, respectively (Fig. 2d; Tukey's test p < 0.05). In contrast, establishment rates with the heat-sensitive type C1 decreased with increasing temperature ($F_{3\,6}$ = 26.210, p = 0.001). At 25%, 74.4 ± 12.2% of larvae acquired type C1 symbionts, and this decreased to 16.7 ± 4.2% at 31°C (Fig. 2e). Temperature did not significantly alter uptake patterns of type A3 in *A. monticulosa* (Fig. 2f).

Densities of *Symbiodinium* cells within infected larvae were also strongly dependent upon temperature and the thermal tolerance of the symbiont type. At 32°C, there were significantly higher densities of the heat-tolerant type A in *A. millepora* compared to any other temperature (Fig. 3a; $F_{4,127}$ = 4.583, p = 0.01; Tukey's test p < 0.05). Densities of the heat-sensitive symbiont type C1 at each light level depended on the prevailing temperature ($F_{4,161}$ = 0.658, p < 0.001). Namely, at high light levels symbiont densities were highest at 26°C, while under low light this shifted to 28°C (Fig. 3b). Importantly, symbiont densities significantly dropped at 30 and 32°C (Tukey's test p < 0.05), and were similarly low irrespective of the light level. For example, at 32°C there were only 2 cells occurring within the infected larvae (Fig. 3b). Densities of the heat-sensitive type A3 in *A. millepora* were significantly lower at 32°C regardless of light levels ($F_{4,132}$ = 21.489, p < 0.001; Tukey's test p < 0.05). At temperatures ranging from 25–30°C, densities exceeded 270 cells, but this dropped dramatically to below 15 cells at 32°C (Fig. 3c). Similar cell density patterns were observed in *A. monticulosa* larvae. The heat-tolerant type A symbionts had the highest cell density in *A. monticulosa* larvae exposed to the highest temperature of 31°C (Fig. 3d). However, while the ANOVA suggested a significant effect of temperature, Tukey's test did not reveal a significant difference. Densities of the heat-sensitive type C1 cells were significantly lower at 31°C compared to 25 and 28°C ($F_{3,21}$ = 39.144, p < 0.001; Tukey's test p < 0.05). In fact, there were only an average of 2 cells per infected larva exposed to 31°C,

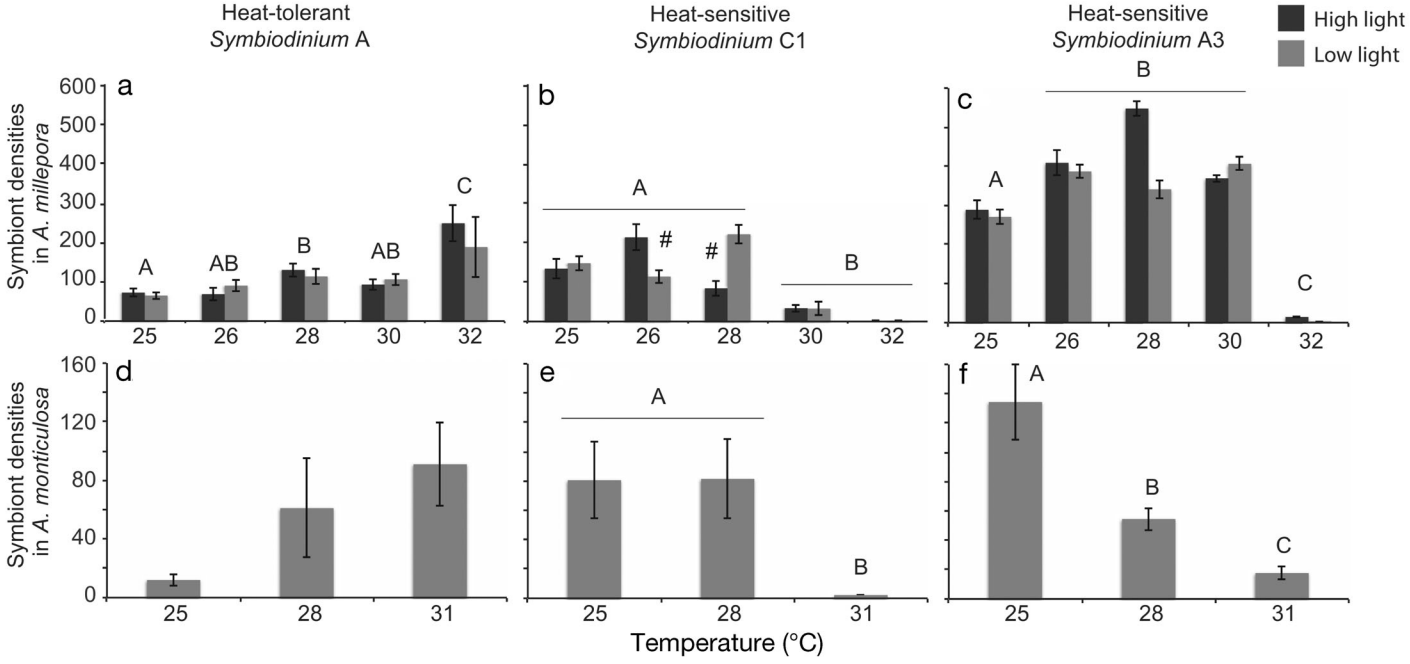

Fig. 3. Densities (mean ± SE) of (a) heat-tolerant *Symbiodinium* type A, (b) heat-sensitive type C1, and (c) heat-sensitive type A3 in *Acropora millepora* larvae (top row) after 6 d of exposure to temperatures of 25°C, 26°C, 28°C, 30°C, and 32°C, and a low and high light. Similarly, densities of the same *Symbiodinium* types in *A. monticulosa* (bottom row) after 6 d of exposure to 25, 28 or 31°C. Significant interactions between temperature and light are indicated with a hash (#). Significant differences (Tukey post hoc test; p < 0.05) among temperatures are denoted by letters above bars

while there were approximately 81 cells per infected larva exposed to 25 and 28°C (Fig. 3e). Similarly, densities of the heat-sensitive type A3 were significantly lower at the highest temperature ($F_{3,45}$ = 140.286, p < 0.001; Tukey's test < 0.05), with only 18 cells in the larvae compared to 134 and 55 cells at 25 and 28°C, respectively (Fig. 3f).

DISCUSSION

Temperature had a strong effect on the establishment and development of symbiosis, and this effect was predictably dependent on the thermal tolerance of free-living *Symbiodinium*. Under elevated temperature, larvae successfully established symbiosis with the heat-tolerant *Symbiodinium*, and their densities were greatest. Conversely, establishment and development of symbiosis with the heat-sensitive symbiont types were greatly diminished at the highest temperatures (Figs. 2 & 3). Therefore, with projected increases in temperatures, successful establishment and development of symbiosis with heat-sensitive types is likely to decline. We predict that climate-driven incremental rises in SSTs will result in changes in symbiont associations between generations to partners more suited to the new environment, thereby providing a mechanism by which symbiotic organisms with horizontal transmission can adjust to climate change.

Generally, high irradiance coupled with high temperatures increases photoinhibition in *Symbiodinium* (Bhagooli & Yakovleva 2004), and because of this stress response, we expected lower levels of establishment and development of symbiosis in the high light treatment at high temperatures compared to the low light treatment. Surprisingly, the prevailing irradiance level did not have a strong effect on the patterns of establishment and development of symbiosis in *Acropora millepora* (Figs. 2 & 3). High light coupled with high irradiance resulted in lower establishment of type A3 only (Fig. 2c). Our results are consistent with Abrego et al. (2009b), who demonstrated that light does not affect the *Symbiodinium* types that established symbiosis with juvenile *Acropora* spp. A 4-fold difference in light environment in the field (high = 540 µmol quanta m^{-2} s^{-1} and low = 140 µmol quanta m^{-2} s^{-1}) had no effect on the initial association in *A. millepora* and *A. tenuis* juveniles, with both species dominated by type D regardless of the light environment (Abrego et al. 2009b).

Whether heat-tolerant symbionts are retained throughout the life of a coral remains an open question. While some coral species can acquire multiple symbiont types as larvae or juveniles (Byler et al. 2013, Cumbo et al. 2013), a winnowing process (Nyholm & McFall-Ngai 2004) occurs through ontogeny until adult colonies are dominated by 1 *Symbiodinium* type (LaJeunesse 2001, Goulet 2006) or genotype (Baums et al. 2014). Winnowing is thought to begin during initial cell recognition (Rodriguez-Lanetty et al. 2006, Bay et al. 2011) and continues through competition among different symbionts inside the host (Dunn & Weis 2009, Abrego et al. 2012) and active selection by the host to maximise symbiont effectiveness (Little et al. 2004). Generally, at a specific location and therefore environment, the symbiont type that eventually dominates a host is the dominant type in adult corals of that species (Coffroth et al. 2001, Abrego et al. 2009b, Byler et al. 2013). However, symbiont types within species differ among locations that have different environments (Rodriguez-Lanetty et al. 2001, Toller et al. 2001, LaJeunesse et al. 2004, 2010, Hume et al. 2015) or within a location after stress events (Baker et al. 2004, Cunning et al. 2015). Therefore, when a permanent difference in environmental conditions exists, the winnowing process is altered to favour a different host–symbiont combination. We predict that projected incremental rises in SST will also alter this ontogenetic winnowing of symbionts, and the symbiont most beneficial for coral survival will be retained (Fig. 4). If novel symbiont types are retained, a shift in the structure of coral–*Symbiodinium* assemblages on reefs will occur (Pettay & LaJeunesse 2013). Indeed, the successful colonisation by corals of the extreme environment in the Persian Gulf in recent history is hypothesized to have been mediated by the acquisition of novel and thermally resistant symbiont types (Hume et al. 2016).

Predicting the fate of such novel symbioses requires consideration of the trade-offs between increasing thermal tolerance and other life history traits, such as growth, survival and fecundity (Mieog et al. 2009, Jones & Berkelmans 2010, Sampayo et al. 2016). Most studies have predicted limited long-term benefits of associating with heat-tolerant *Symbiodinium* because they provide less energy to the coral host (Cantin et al. 2009), thereby reducing growth (Little et al. 2004) and, presumably, increasing mortality. However, these trade-offs could diminish as temperatures increase. For example, at ambient temperatures, *Pocillipora damicornis* colonies hosting clade C symbionts grew 40% faster than those hosting members of the heat-tolerant clade D (Cunning et al. 2015). However, at elevated temperatures, colonies hosting the heat-tolerant clade D had similar growth rates to those hosting

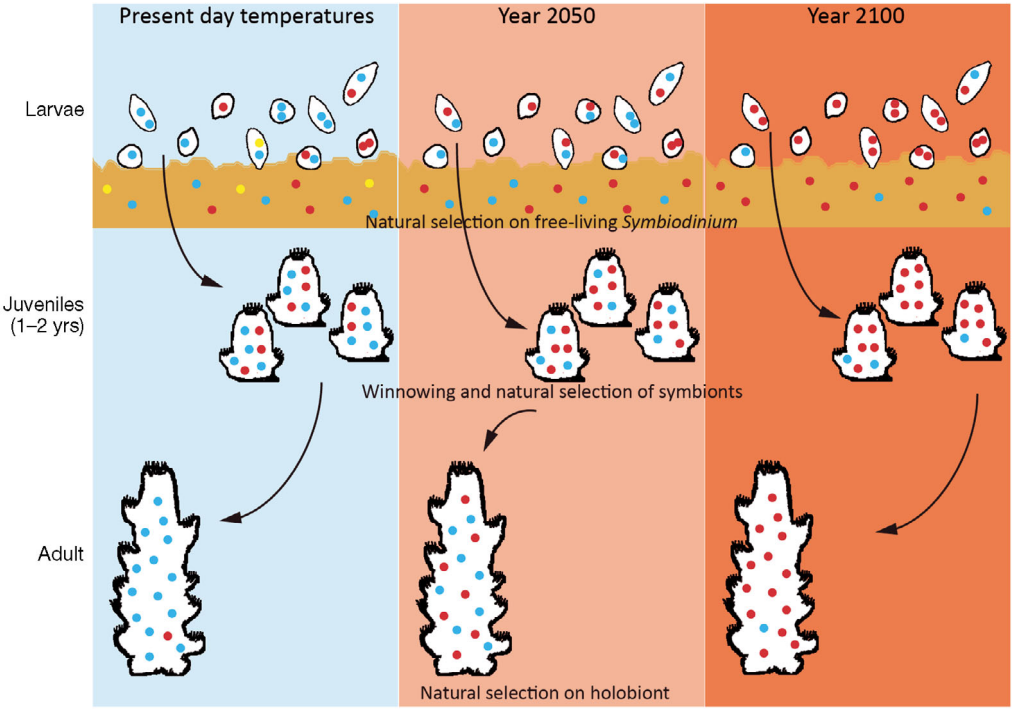

Fig. 4. Predicted changes in free-living and symbiont *Symbiodinium* communities with projected rises in sea surface temperatures. Multiple free-living *Symbiodinium* types are acquired by coral larvae under present-day temperatures (yellow dots = most heat-sensitive type, blue dots = heat-sensitive type, red dots = heat-tolerant type). A winnowing process occurs through ontogeny until adult colonies are dominated by 1 *Symbiodinium* type. Under projected elevations in temperature (Year 2050 and 2100), the free-living *Symbiodinium* assemblage is altered, with some types becoming extinct, and this alters patterns of establishment of symbiosis. When a permanent difference in environmental conditions exists, the winnowing process is also altered to favour a different host–symbiont combination

clade C (Cunning et al. 2015). Importantly, at elevated temperatures, corals containing the heat-tolerant symbionts had very low whole colony and partial mortality (<6%) unlike those hosting clade C, where 39% of colonies died and 24% suffered major partial mortality (Cunning et al. 2015). Therefore, with projected elevations in temperature, the growth and survival of hosts that acquire heat-tolerant symbionts during establishment of symbiosis are likely to be higher than for hosts with heat-sensitive symbionts, resulting in higher retention of these types within the symbiont population (Fig. 4).

Acquisition of symbionts via horizontal transmission relies on an environmental source of *Symbiodinium.* Projected environmental change will also affect this free-living *Symbiodinium* source, and any change in this source assemblage (i.e. the relative abundance of specific types, change in *Symbiodinium* health or acclimatisation of the symbionts) could alter patterns of establishment of symbiosis, and the resulting coral–symbiont assemblage structure (Fig. 4). While our study showed that the photosynthetic efficiency of the heat-sensitive *Symbiodinium* types in culture was unaffected by temperatures up to 31°C (Fig. 1b,c), the establishment and proliferation of these types within the host at similar temperatures was greatly diminished (Figs. 2 & 3). Elevated temperatures are likely affecting other factors such as their host infectivity and growth rate, resulting in this decline. A potential ecological example of an environmentally driven change in the free-living *Symbiodinium* assemblage is the extinction of a type around Magnetic Island on the GBR. While *Symbiodinium* C2 is the most prevalent symbiont type in corals at most locations on the GBR (van Oppen et al. 2001, LaJeunesse et al. 2003, LaJeunesse 2005), it is absent or extremely rare in colonies at Magnetic Island (Ulstrup & Van Oppen 2003, Abrego et al. 2009a). *Symbiodinium* C2 is a light-loving symbiont (Ulstrup & Van Oppen 2003), and the waters around Magnetic Island are on occasion highly turbid. Turbidity, coupled with high temperatures and low salinity events that are frequent at this location (Berkelmans 2002), may have resulted in its reduced abundance. If certain *Symbiodinium* types become locally extinct, or too unhealthy for uptake due to

elevated temperatures, a shift in the types establishing symbiosis with corals is inevitable.

Previous research into potential mechanisms by which organisms might adjust to climate change has focussed on within-generation symbiont switching in response to acute disturbances, such as high temperature and irradiance (Baker 2001, Kinzie et al. 2001, Kiers et al. 2010). However, in adult corals these changes are not always stable, with *Symbiodinium* assemblages reverting back to pre-disturbance structure when temperatures return to normal (Thornhill et al. 2006, Coffroth et al. 2010, Sampayo et al. 2016). While within-generation symbiont-driven responses to environmental change do occur in a range of insect–microbe symbioses, increasing the range of habitats that these co-living organisms can tolerate (Dunbar et al. 2007, Wernegreen & Wheeler 2009, Mueller et al. 2011), switching symbionts in other systems can physiologically stress the hosts (reviewed by Kiers et al. 2010). Our results offer an alternative mechanism for rapid adjustment to changing environments, namely a change in the symbiont type between generations during the initial establishment of symbiosis (Fig. 4). Flexibility in symbiont association is potentially a feature of the life history of every organism that acquires its symbionts anew in each generation. Each episode of sexual reproduction therefore provides the opportunity for the symbiosis to change in response to the prevailing environmental conditions. Our results demonstrate that changes in the establishment of a symbiont are dependent on its thermal tolerance and the prevailing temperature. Therefore, rising temperatures will likely induce the establishment of thermally tolerant symbioses that are better suited to the changed environmental conditions they encounter (Fig. 4). Our results suggest an increase in the prevalence of species with heat-tolerant symbionts with further climate warming due to this transgenerational, environmentally mediated change in host–symbiont partnerships.

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