Impact of Light and Temperature on the Uptake of Algal Symbionts by Coral Juveniles

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Abstract
The effects of temperature and light on the breakdown of the coral-Symbiodinium symbiosis are well documented but current understanding of their roles during initial uptake and establishment of symbiosis is limited. In this study, we investigate how temperature and light affect the uptake of the algal symbionts, ITS1 types C1 and D, by juveniles of the broadcast-spawning corals Acropora tenuis and A. millepora. Elevated temperatures had a strong negative effect on Symbiodinium uptake in both coral species, with corals at 31 °C showing as little as 8% uptake compared to 87% at 28 °C. Juveniles in high light treatments (390 μmol photons m⁻² s⁻¹) had lower cell counts across all temperatures, emphasizing the importance of the light environment during the initial uptake phase. The proportions of the two Symbiodinium types taken up, as quantified by a real time PCR assay using clade C- and D-specific primers, were also influenced by temperature, although variation in uptake dynamics between the two coral species indicates a host effect. At 28 °C, A. tenuis juveniles were dominated by C1 Symbiodinium, and while the number of D Symbiodinium cells increased at 31 °C, they never exceeded the number of C1 cells. In contrast, juveniles of A. millepora had approximately equal numbers of C1 and D cells at 28 °C, but were dominated by D at 30 °C and 31 °C. This study highlights the significant role that environmental factors play in the establishment of coral-Symbiodinium symbiosis and provides insights into how potentially competing Symbiodinium types take up residence in coral juveniles.

Introduction
Many marine cnidarians form symbioses with dinoflagellates of the genus Symbiodinium, gaining significant nutritional resources that underpin the capacity of scleractinian corals to build coral reefs [1,2]. The increasing frequency and severity of abnormally high seawater temperatures in recent decades have led to both localized and widespread mass coral mortalities and phase shifts on some coral reefs [3,4], highlighting the need to understand the effects of changing environmental factors on coral-Symbiodinium symbioses. Some types of Symbiodinium (9 clades are currently recognized, each comprising multiply types [3]) typically confer bleaching resistance, particularly types in clade D, as deduced from changes in symbiont communities during or shortly after bleaching events [6-9] but see [10]. In addition, variation in the physiological responses of different Symbiodinium types to temperature and light is well documented, both in culture and in hospite [10-16]. However, the effects of temperature and light on the initial uptake and establishment of symbiosis are poorly known.

In most coral species (>85%), Symbiodinium endosymbiosis is established with each new generation by acquisition of Symbiodinium from the environment, i.e., horizontal transmission [17-19]. A number of studies of Symbiodinium uptake at ambient, non-stressful temperatures, either in the field or in controlled experiments, have shown that corals and other cnidarians can be infected by Symbiodinium types different from those previously detected in their tissues or those found in parental colonies [12,20-27]. However, there is a gap in current understanding about whether such flexibility is likely to persist and provide options for survival of the coral holobiont during stressful environmental conditions, such as those expected to become more common over the next decades [28]. Only three studies have investigated the onset of symbiosis in broadcast spawning corals at stressful temperatures, and all have focused on Symbiodinium uptake in larvae [29-31]. The pattern emerging is that uptake of algal symbionts by larvae is lowest at high temperatures (>29 °C); moreover, larvae associated with Symbiodinium have lower survival rates at high temperatures than those without symbionts. However, it is not known if high temperatures similarly affect establishment of symbiosis in coral juveniles following metamorphosis, an ecologically important stage for Symbiodinium uptake, given evidence of order of magnitude greater abundances of Symbiodinium in reef sediments compared to the water column [32].

In corals with horizontal symbiont transmission, more than one type of Symbiodinium, including non-homologous types, may be taken up over a relatively short period of time at the larval or primary polyp stage. We hypothesize that intrinsic physiological differences between Symbiodinium types may confer competitive advantages to some types under different thermal regimes, thus the endosymbiont community established may vary with the thermal
Materials and Methods

Experimental design

Tiles with two week-old coral juveniles were placed in three temperature (28 °C, 30 °C, 31 °C) and two light treatments (Low light: 180 μmol photons m⁻² s⁻¹; and High light: 390 μmol photons m⁻² s⁻¹) in temperature-controlled rooms at Orpheus Island Research Station (OIRS). Light levels were chosen to reflect the high turbidity environment of Magnetic Island, where the parental colonies (and sources of Symbiodinium) were collected. Light levels on the reef at Magnetic Island can range from 50–300 μmol photons m⁻² s⁻¹ [36,37]. The photoperiod was 10 hrs light: 14 hrs dark. Within each of the six temperature by light treatments, tiles with corals were haphazardly assigned to four replicate containers supplied with flow-through filtered seawater (1 μm). The number of settled juveniles on each tile varied but approximately the same number of juveniles was allocated to each treatment. The volume of water in the containers was 7 L and flow rate into the containers was approximately 0.5 L minute⁻¹. A small airstone in each container provided a constant stream of micro-bubbles throughout the experiment. Corals were maintained in these temperature by light treatments for 20 (A. tenuis) or 30 days (A. millepora). This difference was due to operational constraints at OIRS, which could not accommodate a 30 day experiment when juveniles of A. tenuis were available.

To explore the differential uptake of Symbiodinium in coral juveniles, types C1 and D were added simultaneously to containers every day during the first half of each experiment. The density of symbionts added to containers ranged between 2.3–6.7 × 10³ cells mL⁻¹. The density varied because symbionts were freshly isolated prior to each inoculation. However, C1 and D symbiont densities were equalized and added in the same volumes to each container. Thus, while the total number of cells added to the containers was not the same at every inoculation, the proportion of C1 and D cells going into each container was always 1:1 and the number of cells going into each container was the same at every inoculation. Water flow through the containers was stopped immediately before adding Symbiodinium and containers were maintained as static cultures for 16–18 hours. After this incubation period, flow was restored to flush containers with new seawater for approximately 6 hours before inoculations were repeated. Corals were maintained in filtered seawater (1 μm) throughout the experimental period. No Symbiodinium cells were added after the half-way point in each experiment in order to explore symbiont dynamics during the establishment phase, when both Symbiodinium types were present.

Effects of temperature and light on Symbiodinium uptake

To assess the impact of temperature and light on the combined uptake of the two Symbiodinium types, the number of juveniles in each treatment was counted on the last day of Symbiodinium inoculation (day 10 for A. tenuis and day 15 for A. millepora) and the level of uptake assessed visually. Each juvenile was assigned to one of two categories (more categories would have introduced subjective bias); juveniles were scored as white when no pigmentation was visible under a dissecting microscope (see Fig. 1), or pigmented when the oral disc, tentacles or walls of juvenile polyps exhibited pigmentation (Fig. 1, see Table 1 for sample sizes). Qualitative visual scoring was repeated on the last day of the experiment (day 20 for A. tenuis and 30 for A. millepora), when well over 100 juveniles remained in almost all of the 24 containers. Counts for white and pigmented juveniles were used to calculate the proportion of juveniles with visible pigmentation in each container (number of pigmented juveniles divided by the total number of juveniles in the container) as a rough measure of uptake efficiency, hereafter referred to as pigmentation ratio. In addition,
the relative survival of juveniles in each temperature by light treatment was calculated based on the number of surviving juveniles at the end of the experiment relative to the number of juveniles at the end of the inoculation phase (mid-point of the experiment). The number of juveniles sub-sampled between the mid- and end-point censuses was subtracted from the total to avoid underestimating relative survival.

To quantify symbiont uptake, samples of *A. tenuis* were collected and the number of *Symbiodinium* cells within each juvenile counted. Twelve samples were collected from each treatment (3 for each replicate container) on days 2, 10, and 20 of the experiment. Juveniles were randomly selected and carefully scraped off tiles using a scalpel blade and fixed in 10% formaldehyde in FSW. Samples were decalcified overnight in 5% formic acid. The acid was removed and the sample was homogenized in 300 μL of milli-Q water using a small dispersion tool on a bench top homogenizer at maximum speed. Prior to homogenization, 30 μL of Alcian Blue dye were added to the sample to facilitate cell counting by pigmenting cell walls. Pigmented samples were kept on the bench for 15 minutes before mixing well with a micropipette and loading 10 μL of mixtures into a haemocytometer chamber. Each sample was counted 10 times. The number of cells was normalized to the number of polyps in each sample (recorded when the samples were scraped off the tiles).

**Effect of temperature and light on the type of *Symbiodinium* acquired and maintained by coral juveniles**

To quantify whether the establishment of *Symbiodinium* types C1 and D occurred in equal ratios in juveniles in each of the six temperature by light treatments, coral juveniles were sub-sampled every other day during the experiment. At each sampling, 10 (A. *millepora*) or 20 (A. *tenuis*) juveniles were randomly sampled from each treatment and fixed in absolute ethanol. Higher initial numbers of *A. tenuis* enabled the higher sample numbers for this species. Extraction of total (coral and algal) DNA from each sub-sample followed a cetyltrimethyl ammonium bromide (CTAB)-based protocol modified from Hoarau et al. [38]. Briefly, each sample was placed in 250 μL of extraction buffer (2% CTAB, 0.1 M Tris (pH 9), 20 mM EDTA (pH 9), 1.4 M NaCl) and macerated inside a 1.5 mL micro-centrifuge tube using a disposable paper clip. The sample was incubated overnight at 60°C followed by addition of 250 μL of chloroform/isoamyl-alcohol (24:1). After thorough mixing, the sample was centrifuged for 15 minutes at maximum speed in a bench top centrifuge. The aqueous phase was pipetted into a new tube and 250 μL of ice-cold 2-propanol was added followed by gentle mixing. The sample was incubated at –20°C for 20 minutes followed by centrifugation at maximum speed at room temperature for 20 minutes. The supernatant was discarded and the pellet washed with 150 μL of 70% ethanol followed by centrifugation for 5 minutes at maximum speed. The pellet was air-dried for 5 minutes and allowed to resuspend overnight in 400 μL of 0.01 M Tris (pH 9).

To quantify the relative abundance of *Symbiodinium* C1 versus *Symbiodinium* D established under different temperature-light treatments, two μL of DNA template were used for a real-time PCR assay, using C- and D-specific ITS1 primers developed by Ustrup and van Oppen [39]. The real-time PCR reaction, containing 10 μL Sybr-Green Super Mix (Invitrogen), 2 μL ITS1 universal forward primer (180 nM), 2 μL ITS1 C or D reverse primer (180 nM), 4 μL of milli-Q water, and 2 μL of DNA template, was run on a Rotor Gene 3000 (Corbett Research). The reaction profile (following an initial heating step to activate the Taq polymerase as per manufacturer’s recommendation) consisted of 40 two-step cycles of 15 s at 95°C and 30 s at 60°C. A melt curve was generated at the end of each run starting at 60°C and ramping to 95°C by increasing 0.5°C every five seconds (except for the first step, which was held for 45 seconds). Data acquisition took place during the 60°C step in each cycle, as well as during the melt curve period. The cycle-threshold (\(C_{T}\)) was set to a fixed value for all runs to allow comparisons between runs. All reactions were run in duplicate. No-template controls and positive controls were included in every run. Data were collected using the Rotor Gene software (v 6.1). The relative abundance (cell ratios) of each *Symbiodinium* type was calculated by the 2-ΔΔ \(C_{T}\) method, taking into account a difference in copy number between clades C and D of three (3 copies of D for every C), as described in Mieog et al. [40]. Cell ratios (D:C ratios) were converted to proportions whereby 1 represented a *Symbiodinium* D only sample with no background of C and 0 represented a C sample with no background of D *Symbiodinium*.

**Table 1. Summary of total number of juveniles counted at each temperature by light treatment during the mid-experiment census (mid) and for the census at the end of the experiment (end).**

| Temperature | High light treatment | Low light treatment |
|-------------|----------------------|---------------------|
|             | n (mid) | n (end) | n (mid) | n (end) |
| *Acropora tenuis* |      |       |       |       |
| 28°C        | 2350    | 1316   | 2943   | 1844   |
| 29°C        | 2394    | 1279   | 2294   | 1050   |
| 31°C        | 2682    | 1064   | 2146   | 663    |
| *A. millepora* |      |       |       |       |
| 28°C        | 354     | 59     | 319    | 121    |
| 30°C        | 147     | 7      | 235    | 73     |
| 31°C        | 180     | 40     | 271    | 84     |

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Data analysis
*Symbiodinium* cell counts and pigmentation ratios were log transformed and analyzed by three-factor ANOVA, with light (2 levels), day (2 levels for pigmentation ratio, 3 for cell counts), and temperature (3 levels) as fixed factors. Homogeneity of variance and normality were verified by Levene’s test, spread vs. residual plots, and Q-Q plots. Pigmentation ratios for *A. millepora* juveniles were analyzed by Kruskal-Wallis test (temperature) and Mann-
Whitney U test (light) as these data did not meet the assumptions of ANOVA after transformation. Relative survival was analyzed by two-way ANOVA with light (two levels) and temperature (3 levels) as fixed factors. Mann-Whitney U and Kruskal-Wallis tests were used for A. millepora juveniles as described above as these data did not meet assumptions of ANOVA. The effects of temperature and light on the D:C cell ratios through time were analyzed by a two factor repeated measures ANOVA. Time (day) was treated as the within subject factor and temperature (three levels, fixed) and light (two levels, fixed) were treated as between subject factors. The assumption of sphericity was checked by Mauchly’s test. All analyses were performed using SPSS software v. 16.0.

Results

Effects of temperature and light on the onset of symbiosis

Qualitative visual assessments of coral juveniles indicated that elevated temperature had a strong negative effect on the uptake and establishment of Symbiodinium cells in both coral species. At the end of the inoculation phase (day 10), the proportion of pigmented juveniles differed significantly between the 28°C and 31°C treatments for Acropora tenuis, with up to four times more juveniles showing signs of Symbiodinium uptake at 28°C (Fig. 2a, c, ANOVA, p<0.001, Table 2). This difference was magnified further by the end of the experiment, when the proportion of juveniles with pigmentation was ten-fold greater at 28°C than at 31°C (Fig. 2c).

The impact of light on the proportion of pigmented juveniles was only evident at 30°C, with A. tenuis juveniles in the low light treatment having significantly higher pigmentation ratios than those in the high light treatment (Fig. 2a, c, ANOVA, p<0.037, Table 2). These qualitative assessments were confirmed by a steady increase in cell counts over time in juveniles of A. tenuis at 28°C, resulting in significantly higher cell counts than found in the two higher temperature treatments (Fig. 3, ANOVA, p<0.046, Table 3). Conversely, there was a declining trend in mean cell counts in the two higher temperature treatments between day ten and day twenty.

Table 2. ANOVA results comparing the pigmentation ratio in A. tenuis juveniles exposed to three temperatures (28, 30, or 31°C) by two light levels (390 µmol photons m⁻² s⁻¹ or 180 µmol photons m⁻² s⁻¹) over a period of 20 days.

| Factor          | Degrees of freedom | F     | Sig.     | Tukey’s |
|-----------------|--------------------|-------|----------|---------|
| Day             | 1                  | 39.975| <0.001   |         |
| Light           | 1                  | 3.595 | 0.068    |         |
| Temp            | 2                  | 1.872 | <0.001   | 28>30>31|
| Day × Light     | 2                  | 0.305 | 0.585    |         |
| Day × Temp      | 2                  | 2.731 | 0.083    |         |
| Light × Temp    | 2                  | 3.707 | 0.037    |         |
| Light × Temp × Day | 2           | 0.762 | 0.476    |         |

Samples were taken on days 10 and 20. Fixed factors were Day, Light, and Temperature. Significant differences were further explored by Tukey’s test. doi:10.1371/journal.pone.0050311.t002

Figure 2. Pigmentation ratios (±1 SE) of coral juveniles kept at 28, 30, or 31°C and under high light (a,b; 390 µmol photons m⁻² s⁻¹) or low light (c,d; 180 µmol photons m⁻² s⁻¹) levels. Pigmentation ratios were calculated after 10 (A. tenuis a, c) or 15 (A. millepora b, d) days of exposure to Symbiodinium, and again after a further 10 or 15 days in filtered sea water (1 µm) without additional exposure to Symbiodinium. See Table 1 for sample sizes. doi:10.1371/journal.pone.0050311.g002

Figure 3. Symbiodinium cell counts in A. tenuis juveniles kept at 28, 30, or 31°C and under high light (a, 390 µmol photons m⁻² s⁻¹) or low light (b, 180 µmol photons m⁻² s⁻¹) levels. The number of cells was calculated from 10 replicate counts for each of 12 samples per treatment per day. Cell counts (±1 SE) were normalized to the number of polyps taken by each sample. doi:10.1371/journal.pone.0050311.g003
Effects of temperature and light on type of symbiont acquired and maintained

Elevated temperatures had the overall effect of significantly increasing the D:C cell ratio in juveniles of both coral species by the end of the experimental period (Tables 4–5), regardless of the light level (Figs. 5a–b). For juveniles of A. tenuis, Symbiodinium communities in all treatments were initially dominated by type C1 (D:C cell ratios <0.5, Figs. 5a–b). D:C cell ratios decreased initially during the inoculation phase (first 10 days) and were very similar across all temperature and light treatments. However, once Symbiodinium inoculations ceased half way through the experimental period, D:C cell ratios increased in juveniles at 30°C and 31°C but not at 28°C (Figs. 5a–b). This pattern was similar for juveniles of A. tenuis in both light levels until the end of the experiment, when the D:C ratio in corals at 30°C reached levels more than two-fold higher than those at 31°C (although they remained below 0.5, Figs. 5a–b).

Initially, Symbiodinium communities in juveniles of A. millepora were dominated by type C1 at high light levels for all temperatures (Fig. 6a). However, unlike A. tenuis, D:C cell ratios increased during the inoculation phase, reaching approximately equal densities by the end of this period (day 15, Fig. 6a). D:C cell ratios in the higher temperature treatments continued to increase after Symbiodinium inoculations ceased, but the D:C cell ratio in juveniles at 28°C remained close to 0.5 until the last sampling point, when it reached 0.8, indicating a D-dominated symbiosis (Fig. 6a). For juveniles at the low light level, the D:C cell ratios also increased over time in the 30°C and 31°C treatments but remained at approximately equal densities in the control treatment (28°C, Fig. 6b).

Discussion

This study highlights the importance of temperature and light for the uptake and establishment of Symbiodinium symbioses in coral juveniles. Our results show that elevated temperatures have a significant impact on the establishment of the initial symbiosis in coral juveniles, similar to the responses found for coral larvae to elevated temperatures [29–31]. The low level of Symbiodinium
uptake at the two higher temperatures, in comparison to the 10-fold higher pigmentation ratio and up to 2.6-fold increase in *Symbiodinium* cells in juveniles kept at 28°C, has important implications for coral persistence given projected increases in sea surface temperatures associated with climate change [28]. First, acquisition of *Symbiodinium* by newly settled juveniles of broadcast spawning corals on the Great Barrier Reef occurs during late spring and early summer [41], when seawater temperatures are typically increasing. At Magnetic Island, corals generally spawn in October or November when seawater temperatures typically reach 28°C and continue to warm through February (Fig. 7). Elevated temperatures may compromise the formation of the symbiosis by inducing oxidative stress in both host and algal cells. High levels of superoxide dismutase (SOD) and malondialdehyde (MDA) were detected in coral larvae exposed to 32°C [30]. Moreover, both SOD and MDA levels were more than double in larvae with algal cells than in *Symbiodinium*-free larvae, suggesting that algal cells may become a liability to the host at high temperatures by increasing oxidative stress and ultimately higher mortality [30]. Lower survival of symbiotic compared to non-symbiotic larvae at high temperatures has also been documented by Schnitzler et al [29]. However, low rates of infection due to elevated temperatures may result in low survival of recruits and/or slow growth of juveniles as a consequence of diminished transfer of photosynthates to the coral host [42]. Although patterns of juvenile survival over the time span covered here were not significantly different among temperature treatments, it is unlikely that uninfected juveniles would have survived for longer periods of time, given the fundamental role of algal endosymbiosis in coral survival [1].

Counts of *Symbiodinium* cells in juveniles of *A. tenuis* clearly show that light has a significant impact on the uptake of algal symbionts (Fig. 5). The higher number of symbionts in the low light treatment across all temperatures may be explained by lower levels of oxidative stress on these cells compared to those at high light levels. Under normal conditions, *Symbiodinium* cells are capable of protecting themselves from oxidative damage by non-photochemical quenching [43]. However, these mechanisms can be overwhelmed by high light or high temperature stress, which may explain why *A. tenuis* juveniles at 31°C in the high light treatment had the lowest *Symbiodinium* cell counts. The role of light was also evident by the significant interaction between light and temperature in the qualitative visual assessments of *Symbiodinium* uptake, which was driven by higher pigmentation ratios in juveniles exposed to low light at 30°C than those in high light. Light had no effect on symbiont densities in a previous four-day study using coral larvae maintained in either ambient light levels or virtual darkness [24]. However, these results may have reflected the short duration of the experiment, as a possible lag phase in *Symbiodinium* population growth immediately following inoculation.
Table 4. Repeated measures ANOVA results comparing changes in D:C cell ratios in *Acropora tenuis* juveniles kept at three temperatures (28, 30, or 31 °C) by two light levels (390 μmol photons m⁻² s⁻¹ or 180 μmol photons m⁻² s⁻¹).

| Source of variation | Sum of squares | Degrees of freedom | Mean square | F     | Sig.  | Tukey's |
|---------------------|----------------|--------------------|-------------|-------|-------|---------|
| Within Subjects analysis |                |                    |             |       |       |         |
| Day                 | 0.497          | 9                  | 0.055       | 3.241 | 0.001 |         |
| Day × Light         | 0.380          | 9                  | 0.042       | 2.478 | 0.012 |         |
| Day × Temp          | 0.987          | 18                 | 0.055       | 3.216 | <0.001|         |
| Day × Light × Temp  | 0.317          | 18                 | 0.018       | 1.034 | 0.427 |         |
| Between Subject analysis |              |                    |             |       |       |         |
| Light               | 0.024          | 1                  | 0.024       | 1.229 | 0.286 |         |
| Temp                | 0.758          | 2                  | 0.379       | 19.020| <0.001| 28<30, 31|
| Light × Temp        | 0.084          | 2                  | 0.042       | 2.118 | 0.157 |         |

Within-subjects factor Day was 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20. doi:10.1371/journal.pone.0050311.t004

Table 5. Repeated measures ANOVA results comparing changes in D:C cell ratios in *A. millepora* juveniles kept at three temperatures (28, 30, or 31 °C) by two light levels (390 μmol photons m⁻² s⁻¹ or 180 μmol photons m⁻² s⁻¹).

| Source of variation | Sum of squares | Degrees of freedom | Mean square | F     | Sig.  | Tukey's |
|---------------------|----------------|--------------------|-------------|-------|-------|---------|
| Within Subject analysis |                |                    |             |       |       |         |
| Day                 | 4.874          | 7                  | 0.696       | 33.621| <0.001|         |
| Day × Light         | 0.400          | 7                  | 0.057       | 2.757 | 0.013 |         |
| Day × Temp          | 1.613          | 14                 | 0.115       | 5.565 | <0.001|         |
| Day × Light × Temp  | 1.149          | 14                 | 0.082       | 3.964 | <0.001|         |
| Between Subject analysis |              |                    |             |       |       |         |
| Light               | 0.020          | 1                  | 0.020       | 1.458 | 0.253 |         |
| Temp                | 2.412          | 2                  | 1.206       | 85.858| <0.001| 28<30, 31|
| Light × Temp        | 0.041          | 2                  | 0.020       | 1.456 | 0.275 |         |

Within-subjects factor Day was 2, 6, 10, 14, 20, 23, 27, and 30. doi:10.1371/journal.pone.0050311.t005

Over time in juveniles of both species are consistent with the view that type D is better at infecting and/or replicating within corals at high temperatures. It is important to recognize that while the patterns observed in our study may be the result of competitive interactions between C1 and D symbionts, a similar outcome could result in the absence of direct competition. Type D symbionts may simply thrive in warmer temperatures while C1 do not, thus type D symbionts could colonize space not occupied by C1 symbionts without actually displacing them. Although patterns of greater uptake of type D detected in our study are at odds with results of an earlier study showing that the thermal tolerance of *A. tenuis* juveniles is not enhanced by associating with clade D *Symbiodinium* compared to C1 [10], they are consistent with field observations of *Symbiodinium* type D uptake by coral juveniles (regardless of the type found in adult colonies) a few weeks after annual spawning events, when water temperatures are approaching their highest levels [27]. However, the benefits of associating with *Symbiodinium* D to the coral host remain to be fully explored given that juveniles at high temperatures had low pigmentation ratios and *Symbiodinium* cell numbers and may therefore be photosynthetic-limited. This likely impedes rapid growth of juvenile colonies and makes them more prone to
pitfall to compensate for possible low photosynthetic inputs [47].

Species-specific differences in the uptake of *Symbiodinium* documented in this study indicate the potential existence of host effects in the early stages of establishment of symbioses. These differences were particularly evident at the ambient control temperature, where high *Symbiodinium* cell numbers and pigmentation levels indicated successfully established symbioses. In *A. tenuis* juveniles, the association was almost entirely C-dominated by the end of the experiment (D:C ratio < 0.02), as is typical of *A. tenuis* adults at Magnetic Island (i.e. type C1 is homologous). In contrast, in *A. millepora* juveniles at the control temperature, the symbiosis was approximately equal in proportion (D:C ratio of 0.45 at low light), or dominated by type D *Symbiodinium* (D:C ratio of 0.8 at high light). Furthermore, juveniles at 30°C, which also had high pigmentation ratios, were almost completely dominated by type D *Symbiodinium* (D:C ratio >0.93). This is the type found in *A. millepora* adults at Magnetic Island. Thus, the dominance of the homologous *Symbiodinium* type in both coral species at the control temperature shows that host effects play a role in the early establishment of the symbiosis. However, elevated temperatures may disrupt this interaction. *A. tenuis* juveniles at high temperatures had a higher proportion of type D *Symbiodinium* than conspecifics at 28°C. On the reef, uptake of *Symbiodinium* by *A. tenuis* juveniles is dominated by type D [27]. Initial uptake on the reef occurs in early summer, when sea-surface temperatures are rising towards maximum levels (see Fig. 7). Hence, high temperatures in the field may inhibit mechanisms by which host effects play a role in the uptake of homologous symbionts and result in non-specific uptake.

Our study provides insights into how different symbionts take up residence in newly settled corals. Corals are able to acquire *Symbiodinium* as early as the larval stage [22,48], which means that larvae on the reef may be exposed to symbionts before settlement. It is not known whether recruits in the field lack symbionts before settlement or the extent to which corals that have acquired symbionts as larvae are able to do so after settlement. Several studies provide evidence that initial uptake of symbionts by coral juveniles is a dynamic process that can extend for weeks to years before a stable association is formed [23,25–27,35]. Moreover, findings of higher densities of *Symbiodinium* and *Symbiodinium*-like algae in reef sediments than in the water column [32,49,50], along with periodic motile phases observed for *Symbiodinium* in culture, suggest that encounters with settled juveniles on the reef are likely. It is also worth noting that coral larvae do not require contact with *Symbiodinium* in order to settle and metamorphose [31,52].

Inherent physiological differences between the two *Symbiodinium* types may provide a competitive edge to type D at elevated temperatures or may allow this symbiont type to quickly occupy available space in the absence of direct competition with other symbiont types. However, inherent physiological differences alone cannot explain the host-specific patterns in uptake and establishment detected in this study showing that host effects in closely related coral species have significant impacts in early symbiont uptake and establishment of the symbiosis.

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**Author Contributions**

Conceived and designed the experiments: DA BLW MJHvO. Performed the experiments: DA. Analyzed the data: DA. Contributed reagents/materials/analysis tools: BLW MJHvO. Wrote the paper: DA BLW MJHvO.

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