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Intraspecific and interspecific variation in thermotolerance and photoacclimation in *Symbiodinium* dinoflagellates

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Light and temperature are major drivers in the ecology and biogeography of symbiotic dinoflagellates living in corals and other cnidarians. We examined variations in physiology among 11 strains comprising five species of clade A *Symbiodinium*. We grew cultures at 26°C (control) and 32°C (high temperature) over a duration of 18 days while measuring growth and photochemical efficiency (F_v/F_m). Responses to thermal stress ranged from susceptible to tolerant across species and strains. Most strains exhibited a decrease in cell densities and F_v/F_m when grown at 32°C. Tolerance to high temperature (T_{32}) was calculated for all strains, ranging from 0 (unable to survive at high temperature) to 1 (able survive at high temperature). There was substantial variation in thermotolerance across species and among strains. One strain had a T_{32} close to 1, indicating that growth was not reduced at 32°C for only this one strain. To evaluate the combined effect of temperature and light on physiological stress, we selected three strains with different levels of thermotolerance (tolerant, intermediate and susceptible) and grew them under five different light intensities (65, 80, 100, 240 and 443 µmol quanta m^{-2} s^{-1}) at 26 and 32°C. High irradiance exacerbated the effect of high temperature, particularly in strains from thermally sensitive species. This work further supports the recognition that broad physiological differences exist not only among species within *Symbiodinium* clades, but also among strains within species demonstrating that thermotolerance varies widely between species and among strains within species.

1. Introduction

The ongoing burning of fossil fuels has substantially increased atmospheric concentrations of carbon dioxide [1], causing global temperatures to rise at unprecedented rates [2,3]. These changes are negatively impacting the planet's more sensitive biota. Episodes of high sea surface temperature (SST) have increased in frequency and intensity, affecting the health and viability of reef-building corals, thus degrading the ecosystems they build and sustain [4,5]. Temperatures that are 1.0–1.5°C above normal are physiologically stressful and can disrupt the mutualisms between corals and their dinoflagellate endosymbionts, *Symbiodinium*. This leads to the whitening, or 'bleaching', of corals. While severe events cause significant mortality, not all symbiotic corals are equally affected [6–8], even among conspecifics from the same population [9]. Certain populations, as well as particular species and colonies of the same species, exhibit differential responses to heat stress. Often, these differences in coral mortality are in large part due to physiological differences

Table 1. Cultures used in this study, culture collection code, ITS2 type, animal species from which the culture was obtained, and geographical location where the culture was collected.

code	species name	strain name	type	collected from	origin	thermotolerance
Smic1	*S. microadriaticum*	CassKB8	A1[a]	*Cassiopeia xamachana*	North Pacific	tolerant
Smic2	*S. microadriaticum*	RT-061	A1[b]	*Cassiopeia xamachana*	Caribbean	tolerant
Smic3	*S. microadriaticum*	RT-362	A1[b]	*Cassiopeia andromeda*	Red Sea	sensitive
Snec1	*S. necroappetens*	RT-80	A13[b]	*Condylactis gigantea*	Caribbean	tolerant
Snec2	*S. necroappetens*	MAC4-225	A13[a]	*Porites astreoides*	Caribbean	sensitive
Slin1	*S. linucheae*	RT-379	A4[b]	*Plexaura homamalla*	Caribbean	tolerant
Spil1	*S. pilosum*	RT-024	A2[b]	*Bartholomea annulata*	Caribbean	sensitive
Spil2	*S. pilosum*	RT-104	A2[b]	*Heliopora* sp.	West Pacific	tolerant
Spil3	*S. pilosum*	RT-130	A2[b]	*Meandrina* sp.	Caribbean	tolerant
Stri1	*S. tridacnidorum*	RT-292	A3[b]	*Tridacna maxima*	West Pacific	sensitive
Stri2	*S. tridacnidorum*	CassEI-1	A3[a]	*Cassiopeia* sp.	North Pacific	sensitive

[a]BURR collection at SUNY Buffalo.
[b]Trench collection at The Pennsylvania State University.

among the species of *Symbiodinium* they harbour [10–17] and possibly genotypic differences among individual strains.

Physiological and genetic diversity within *Symbiodinium* taxa is vast [14,18,19]. Numerous divergent clades (currently designated A through I) comprise the genus *Symbiodinium* [20] and it is apparent that each clade contains a diversity of species with distinct ecological attributes and physiologies [21–23]. For example, clade C probably contains hundreds of undescribed species that are mutualistic with a wide array of cnidarian hosts [24]. Similarly, there are tens of species within clade B, many of them endemic to the western tropical and subtropical Atlantic [23,25–29]. While the diversity within *Symbiodinium*—one of the most species-rich dinoflagellate genera—is well recognized, the physiological variability within and between species remains poorly characterized.

Stress tolerance among certain *Symbiodinium* may help coral populations cope with rising temperatures [9,30–33]. Tolerance of high temperatures was first observed and described in *Symbiodinium glynnii*, a species in clade D [34–36]. Subsequent studies indicate that *Symbiodinium* spp. from other clades also tolerate higher temperatures [15,33,37,38]. In the Persian Gulf where SSTs can reach a maximum of 34–36°C, several coral species harbour *Symbiodinium thermophilum*, a clade C strain. In the Red Sea, coral reefs experience the most extensive seasonal and latitudinal thermal gradients (21–33°C) worldwide. Along this gradient, the coral species *Pocillopora verrucosa* also exhibit thermotolerant symbionts belonging to clade A (ITS2 type A1 and A21) at higher temperatures (27–33°C) [38]. Beyond interspecies variation, the degree of physiological thermotolerance among strains within populations or among populations across a species' range of distribution remains largely unknown. Individual strains within species of *Symbiodinium* can exhibit different photophysiologies [18], and such variation will factor into the adaptation of *Symbiodinium* to rapid climate change. Thus, it is critical to also assess inter-individual physiological variation.

An important aspect of *Symbiodinium* spp. physiology is their ability to acclimate under physiological stress. For example, members of clade A acclimate differently from one another to various light levels [10–12]. The range of thermal acclimation also differs among *Symbiodinium* spp. [10–12,14,16,17]. Still, the tempo and mode of acclimatory responses within and between most species under the dual effects of light and temperature requires further study.

We conducted experiments to study the acclimation potential to temperature and light within and among species of *Symbiodinium* clade A—a phylogenetically well-characterized group [21,22]. We used 11 cultures from different populations corresponding to five species: *Symbiodinium microadriaticum* (=type A1), *Symbiodinium pilosum* (=type A1), *Symbiodinium tridacnidorum* (=type A3$^{\text{Pacific}}$), *Symbiodinium linucheae* (=type A4) and *Symbiodinium necroappetens* (=type A13). We specifically tested the occurrence of thermotolerance among species, as well as if intraspecific variation is comparable to that found among species. We also evaluated the effects of temperature and light on *Symbiodinium* growth and photochemistry. Our results suggest that thermotolerance is not restricted to individual species, but instead it is widespread throughout clade A, and that species often adjust to variations in light. We also observed that species surviving under stressful light and thermal conditions often incur physiological costs that slow down growth and accelerate photodamage.

2. Methods

(a) *Symbiodinium* cultures: growth conditions

To quantify physiological responses, we cultured *Symbiodinium* in ASP-8A medium [39] at 26°C with full-spectrum fluorescent lights at 100 µmol quanta $m^{-2}\,s^{-1}$ with a 12 : 12 (light : dark) photoperiod. Light intensities were measured inside the culture flasks with a 4π sensor (Biospherical, USA). Cultures were maintained at logarithmic phase by replacing the culture media every 1.5–2 weeks. *Symbiodinium* strains were obtained from the Trench collection maintained by Todd LaJeunesse at The Pennsylvania State University and the BURR collection at SUNY Buffalo maintained by Mary Alice Coffroth. Herein, we follow a binomial nomenclature for *Symbiodinium* species [21,22,40] but table 1 for culture collection and strain designations.

(b) High-temperature acclimation: experimental design

To test for acclimation to high temperature, we used 11 cultures from clade A that included five species: three cultures of *S.*

microadriaticum (ITS2 type A1), two cultures of *S. necroappetens* (ITS2 type A13), three cultures of *S. pilosum* (ITS2 type A2), two cultures of *S. tridacnidorum* (ITS2 type A3^{Pacific}) and one culture of *S. linucheae* (ITS2 type A4) (table 1). We inoculated cultures at an initial concentration of 1×10^6 cells ml^{-1} in 15 ml tubes containing 8 ml of culture (day 0). To elevate the temperature to 32°C, we increased from 28.5°C and then gradually increased the temperature by 0.5°C every 6 h until 32°C was reached (day 2). We counted cell densities in triplicate every 3 days, and measured maximum quantum yield of photochemistry (F_v/F_m) daily (see below) for 17 days in four replicates per strain per temperature (control: 26°C and high temperature: 32°C).

(c) High-temperature acclimation and photoacclimation: experimental design

To test for the effects of interaction between temperature and light on *Symbiodinium* health, we selected three cultures with different levels of known physiological thermotolerance: a tolerant *S. microadriaticum* (*Smic2*) with no significant cell densities changes at 32°C, an intermediate tolerant *S. microadriaticum* (*Smic1*) with significant cell densities changes at 32°C and a sensitive strain from *S. tridacnidorum* (*Stri1*). We inoculated cultures at 1×10^6 cells ml^{-1} in 15 ml tubes, containing 8 ml of culture, initially grown at 100 µmol quanta m^{-2} s^{-1} at 26°C. We set the high-temperature treatment at 32°C (without ramping) and the control temperature treatment at 26°C. Light levels were adjusted to five different intensities ranging from under-saturating levels to over-saturating light levels: 65, 80, 100, 240 and 443 µmol quanta m^{-2} s^{-1}, using mesh fabric to produce shade. We incubated 15 replicates per culture at each of the 10 treatments.

(d) Photochemical efficiency

To quantify photochemical activity, we measured the maximum quantum yield of charge separation of photosystem II (F_v/F_m) with a MiniPAM fluorometer (Walz, USA) after 30–50 min of dark acclimation at the end of the light cycle. We measured daily F_v/F_m in five independent tubes without disturbing the sample. For the species comparisons of 11 strains, these five measurements were performed in at least two independent runs, and in three independent runs for four of these cultures (*Snec1*, *Smic1*, *Stri2* and *Spil1*).

(e) Population growth

To evaluate the performance of 11 strains during high-temperature acclimation, we measured cell density by fixing 1 ml aliquots of culture in 10% Lugol's iodine and determined cell densities using a haematocytometer. At least nine replicate aliquots were counted for each sample at each time point. Cell densities were measured for days 0, 6, 9, 13 and 17. We measured them at least once for all strains; *Smic1*, *Snec1*, *Spil1* and *Stri1* were estimated twice (these strains were chosen randomly).

We used these cell density estimates to estimate relative population growth rates (r, units: d^{-1}) at 26 and 32°C, as well as tolerance to 32°C for each strain. We estimated r as the slope of the relationship between log-transformed cell densities and time, a standard technique for growth experiments with photosynthetic microbes and algae that exhibit exponential growth from low initial densities [41]. To avoid taking the logarithm of zero, we added 111 cells ml^{-1} to each observed count, which is 10% of the smallest observed cell density. We then estimated tolerance to 32°C for each strain, T_{32}, as the ratio of the relative growth rate at 32°C, r_{32}, to the relative growth rate at 26°C, r_{26}. In some cases, strains had negative growth rates at 32°C (i.e. they declined towards extinction). In these cases, we set the tolerance value to a defined minimum value of zero. A tolerance value equal to one indicates that growth is not reduced at 32°C, whereas a value of zero indicates that the isolate cannot grow at this high temperature.

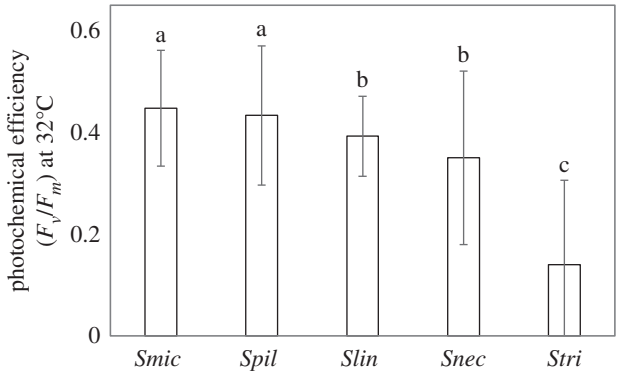

Figure 1. Average photochemical efficiency (F_v/F_m) at 32°C of five *Symbiodinium* spp. over a 17-day period. *Slin*, *S. linucheae* (one strain); *Smic*, *S. microadriaticum* (three pooled strains); *Snec*, *S. necroappetens* (two pooled strains), *Spil*, *S. pilosum* (three pooled strains) and *Stri*, *S. tridacnidorum* (two pooled strains). Bars depict one standard deviation of the mean. (One-way ANOVA, $F_{4,1158} = 191.5$, $p < 0.0001$, Tukey–Kramer HSD.) Grouping from *post hoc* comparisons are depicted with letter above each bar.

(f) Statistical analysis

We examined how cell densities and photochemical efficiency (F_v/F_m) were affected by temperature, comparing *Symbiodinium* strains (11), temperatures (28.5, 29, 29.5, 30, 30.5, 31, 31.5 and 32°C) and time (days 0, 6, 9, 13 and 17). We also analysed the effect of light and temperature, comparing *Symbiodinium* strain (2), temperature (26 and 32°C) and light intensity (65, 80, 100, 240 and 443 µmol quanta m^{-2} s^{-1}). One-way ANOVAs and ANCOVAs (fixed-effects, LS) were used to analyse the effect of temperature and/or light; all two-way interactions were included. We used JMP Pro 11 for all analyses (SAS Institute, Cary, NC, USA, 2014).

We tested for significantly negative effects of high temperature on strain growth using bootstrapping tests (105 iterations, alternative hypothesis: $T_{32} < 1$) in which we randomly resampled data with replacement stratified by each strain, temperature and sampling date [42]. Strains with no thermotolerance are shown in red, while strains with some thermotolerance are shown in a gradient from purple ($T_{32} > 0$) to blue ($T_{32} = 1$).

3. Results

(a) Variation in temperature sensitivity within and among species

Significant differences in F_v/F_m were measured among species (figure 1) and within species (figure 3). Our linear models identified all factors (strain, day, temperature) and two-way interactions as having a significant effect on *Symbiodinium* cell densities and F_v/F_m (table 2 and figures 2 and 3). Temperature × day explained 42.5% of variance in cell densities responses, and 68.1% variance in F_v/F_m values (see F ratios, table 2). The relative population growth rate at 32°C was significantly different among strains (figure 4).

Grouping strains by species (figure 1), we could conclude that there are three groups of species with different thermotolerance: *S. microadriaticum* and *S. pilosum* (group A), *S. linucheae* and *S. necroappetens* (group B) and *S. tridacnidorum* [group C—Tukey–Kramer honestly significant difference (HSD)]. However, fine scale analysis at the level of strains within species showed higher thermotolerance diversity (figures 2–5).

Table 2. ANCOVA analysis examining cell density response ($\log_{10}$ transformed) and photochemical efficiency (F_v/F_m) of 11 strains of dinoflagellates to high temperature. Cell densities and F_v/F_m showed similar results where temperature was the major source of variability. Cell densities were measured on days 0, 6, 9, 13 and 17 in three replicates per culture per treatment ($n = 18$). F_v/F_m was measured every day for 17 days with six technical replicates. F_v/F_m was measured at least two times for all strains and three times for four of them in independent repetitions of the experiment. Cultures were grown at 100 µmol quanta m^2 s^{-1} under two temperatures: control (26°C), and high temperature (32°C). d.f., degrees of freedom.

response	source	d.f.	*F* ratio	Prob > *F*
cell densities (log transformed)	strain	10	264.87	less than 0.0001
	temperature	1	228.32	less than 0.0001
	day	1	2135.78	less than 0.0001
	strain × temperature	10	58.61	less than 0.0001
	strain × day	10	235.38	less than 0.0001
	temperature × day	1	946.77	less than 0.0001
whole model test: $F_{33,2678} = 272.37$, $p < 0.0001$, $R^2 = 0.770$				
photochemical efficiency	strain	10	413.379	less than 0.0001
	temperature	1	534.5921	less than 0.0001
	day	1	3938.295	less than 0.0001
	strain × temperature	10	29.871	less than 0.0001
	strain × day	10	203.1659	less than 0.0001
	temperature × day	1	658.8297	less than 0.0001
whole model test: $F_{33,2278} = 378.1$, $p < 0.0001$, $R^2 = 0.846$				

Using our tolerance estimate, T_{32}, we categorize strains into sensitive ($T_{32} = 0$) or tolerant ($T_{32} > 0$) (table 1 and figure 5). All strains were significantly affected by high temperature (*Spil2* $p = 0.0257$, *Smic2* $p = 0.0006$, $p = 0$ for the rest of the strains). An exception was *Spil3* ($p = 0.3704$), which was not affected by high temperature ($T_{32} = 0.933$). Our thermotolerant strains, in order of tolerance, are *Spil3*, *Spil2*, *Smic2*, *Snec1*, *Smic1* and *Slin1*. The most tolerant strains (*Spil3*, *Spil2* and *Smic2*) showed no significant difference in cell densities ($\log_{10}$ transformed) at high temperature when compared with controls (figures 2 and 5). Only the two most tolerant strains, *Spil3* and *Spil2*, showed no significant difference on F_v/F_m at high temperature compared with control temperature (figure 3). The relative population growth at high temperature was positive for all tolerant strains (figure 4), indicating the ability to grow at high temperature. For the thermotolerant strains *Snec1*, *Smic1* and *Slin1*, cell densities and F_v/F_m were significantly different among treatments indicating a cost in fitness when acclimated to high temperature (figures 2 and 3).

The sensitive strains, in order of thermosensitivity, are: *Stri1*, *Stri2*, *Snec2*, *Smic3* and *Spil1* (figures 2–5). Thermosensitive strains showed negative values of relative population growth (figure 4) indicating the inability to survive at high temperature ($T_{32} = 0$, figure 5). The most thermosensitive strain, *Stri1*, decreased its population size faster than *Stri2* and so on. Sensitive strains *Stri1*, *Stri2* and *Snec2* showed higher sensitivity because cultures died during the experiment. Although cultures of *Smic3* and *Spil1* contained available cells by the end of the experiment (figure 2), the negative trend indicates the culture would likely die if the experiment was conducted for a longer duration (figure 4). All these strains failed to thrive under high temperature, indicating an inability to cope with high temperature.

Species thermotolerance cannot be estimated by most species in this study. This is particularly true for *S. microadriaticum*, *S. necroappetens* and *S. pilosum*. For example, for *S. microadriaticum*, the two strains *Smic1* and *Smic3* decreased in cell density, with *Smic3* showing greater decrease in density than *Smic1* (average 3.9×10^4 cells ml^{-1}, versus 22.6×10^4 cells ml^{-1} at day 17 of the experiment, figure 2). In both cases, F_v/F_m also decreased (figure 3). While cell densities were low for both, these cell cultures were still physiological active (mean F_v/F_m of 0.489 for *Smic1* and 0.310 for *Smic3*). However, the relative population growth rate at high temperature indicates that *Smic3* was negatively affected by high temperature, becoming unable to thrive. By contrast, cell densities of *Smic2* did not change significantly with temperature, although there was a significant decrease in F_v/F_m indicating that it had acclimated better to the heat stress. For *S. necroappetens*, the tolerant *Snec1* strain decreased significantly in cell density and showed a significant decrease in F_v/F_m under high temperature (figures 2 and 3). By contrast, the sensitive strain *Snec2* died under high-temperature conditions. For *S. pilosum*, the cell densities for *Spil1* remained constant until day 13, when growth rates began to decrease (figure 2). Photochemical efficiency for *Spil1* was significantly lower than controls at high temperature (figure 3). Thus, the cells remained physiologically active ($F_v/F_m = 0.534$ versus 0.339), but this activity was insufficient to allow the culture to grow (figure 4). The F_v/F_m values for thermotolerant strains *Spil2* and *Spil3* were not significantly different under high-temperature treatment, and both maintained similar cell densities when grown under control and treatment conditions (electronic supplementary material, table S1; figure 2).

Strains of *S. tridacnidorum* (*Stri1* and *Stri2*) were thermosensitive and both died when exposed to high temperature (figures 2, 4 and 5). Cell densities and F_v/F_m in these cultures decreased soon after the high-temperature treatment was

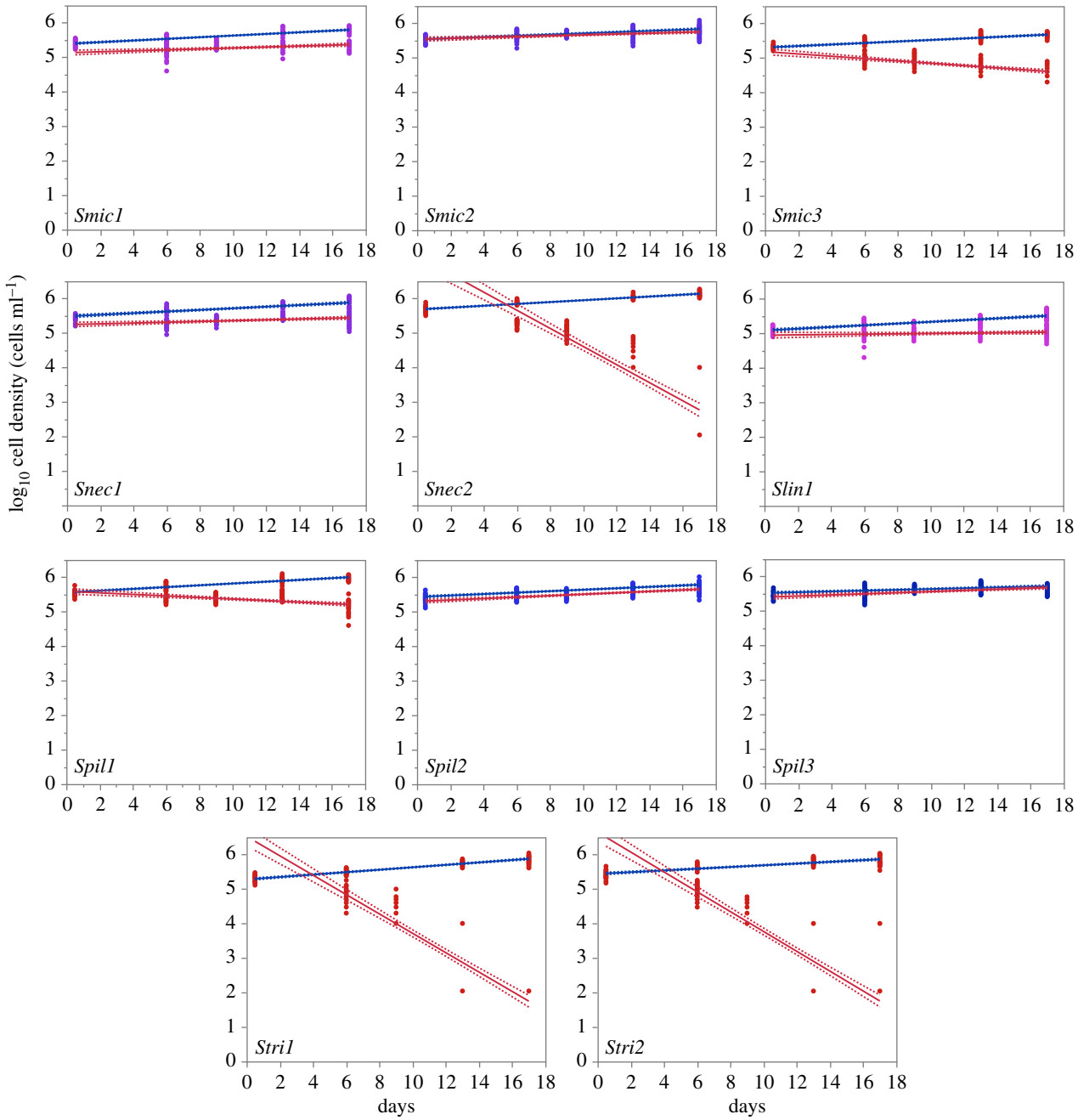

Figure 2. Cell density ($\log_{10}$ transformed) of 11 strains of clade A under different growth temperatures, at 100 µmol quanta m^{-2} s^{-1}. Blue line indicates linear regression of data under control temperature (26°C) and red line indicates linear regression of data under high temperature (32°C). Dotted lines indicate confidence intervals. To avoid taking the logarithm of zero, we added 111, which is 10% of the lowest observed non-zero density, to each value before transformation. A value closer to 2 indicates zero cells. We found that significant differences at the $p < 0.0001$ level between the control and the high-temperature treatments for all species except *Smic2*, *Spil2* and *Spil3*, which showed no statistical difference.

initiated (figure 2). *Symbiodinium linucheae* (*Slin1*) exhibited lower growth rates under the control temperature, compared to other clade A species. The relative population growth rate was positive at high temperature (figure 4), demonstrating its ability to survive under the temperature treatment (figures 2 and 3). For these two species, we cannot categorize them as thermosensitive or thermotolerant, given the limited number of strains, we were able to include in this study.

(b) The synergistic effect of light and temperature in photochemistry and growth

Three strains with different thermal tolerances were chosen to evaluate the synergistic effects of light and high temperature (i.e. *Smic2*, *Smic1* and *Stri1*). Under five different light levels (from low light to high light), all three strains had a linear photoacclimation response independent of temperature (table 3 and figure 6). F_v/F_m was highest at low light and decreased at high light. This linear response had similar slopes for both control and high temperature for all strains. The levels of photochemical efficiency were variable among strains. For the high tolerant strain *Smic2* ($T_{32} = 0.44$), F_v/F_m changed equally under both temperatures and different light intensities. The intermediate tolerant strain *Smic1* ($T_{32} = 0.32$) and sensitive strain *Stri1* ($T_{32} = 0$) decreased F_v/F_m under high temperature but maintained the same slope at both control and high-temperature conditions. The values measured for F_v/F_m decreased at a faster rate for *Stri1* than for *Smic1*. After 7 days of experiment, F_v/F_m remained constant for both tolerant strains. The sensitive

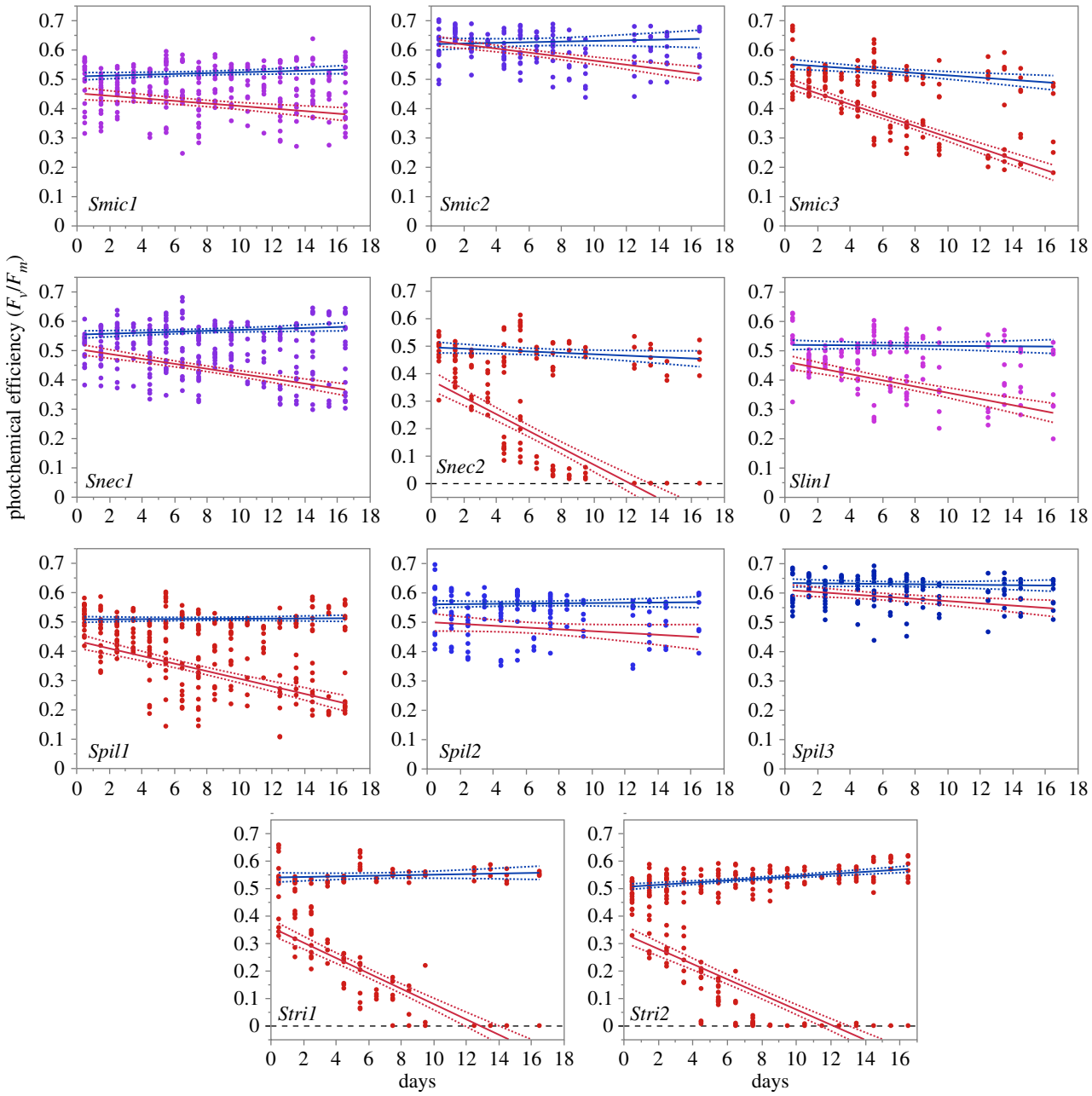

Figure 3. Photochemical efficiency (F_v/F_m) of 11 strains of clade A under different growth temperatures, at 100 µmol quanta m^{-2} s^{-1}. Blue line indicates linear regression of data under control temperature (26°C) and red line indicates linear regression of data under high temperature (32°C). Dotted lines indicate confidence intervals. We found that significant differences at the $p < 0.0001$ level between the control and the high-temperature treatments for all species except *Spil2* and *Spil3*, which showed no statistical difference.

strain under high temperature vastly decreased F_v/F_m and no live cells remained at the end of the experiment (electronic supplementary material, figure S1).

4. Discussion

This work substantiates the growing realization for the existence of broad physiological variation in thermal tolerance among and within species of *Symbiodinium*. Physiologies of strains that are sensitive, intermediate or tolerant appear to be independent of the species under study. There are species comprised strains that are generally thermotolerant (*S. linucheae*), while strains from other species (e.g. *S. tridacnidorum*) are sensitive. Other species within clade A (*S. microadriaticum*, *S. necroappetens* and *S. pilosum*) contain strains that diverge significantly in thermal tolerance. Thus, acclimation to high temperature is dependent on the species identity and the physiological attributes possessed by a particular genotype within a species, i.e. strain. This work also supports previous findings that high light and temperature interact to accelerate physiological breakdown [43], especially among strains with limited or no tolerance to high temperature.

(a) Widespread thermotolerance variation within species from clade A lineage

These findings indicate that at least some strains from each of the clade A species examined here can persist under 32°C, with the possible exception of *S. tridacnidorum*. It appears that thermotolerance is not phylogenetically restricted to certain species, but may indeed be extensive across members of clade A (figure 4). While earlier studies reported limited 'within

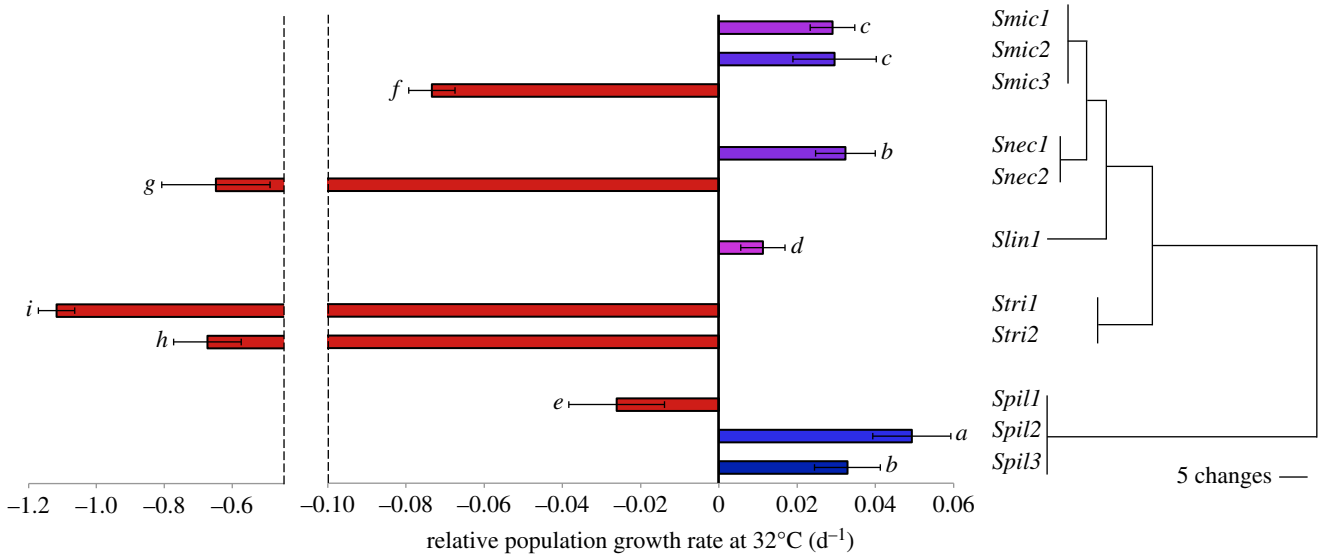

Figure 4. Relative population growth rate at 32°C with simplified phylogeny of *Symbiodinium* clade A inferred from ITS1, ITS2 and 5.8S rDNA sequences [21,22,40]. (One-way ANOVA, $F_{10,310} = 3604.73$, $p < 0.0001$, Tukey–Kramer HSD.) Error bars denote standard error.

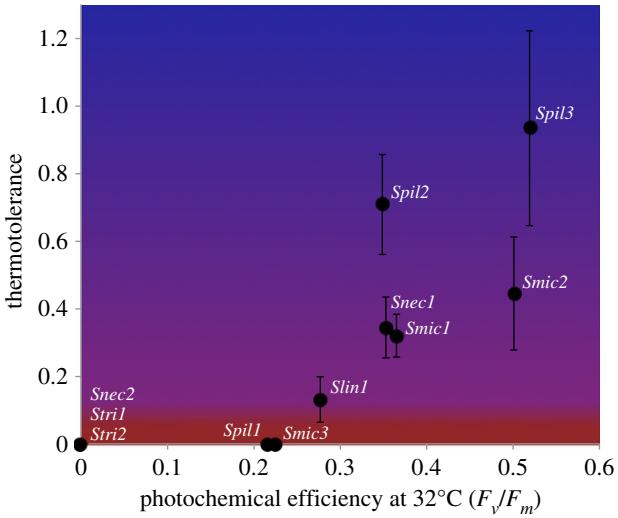

Figure 5. Thermotolerance (T_{32}) versus photochemical efficiency (F_v/F_m) at high temperature (32°C). Error bars denote standard error.

clade' variation in thermal stress [13,14,33,38], our work with additional strains provides evidence for more widespread variation at intraspecific taxonomic levels (i.e. individual strains within species) than previously expected [44].

Extensive variation to thermal tolerance within species hints at genetically encoded variation among populations. Comparing molecular mechanisms at supraspecific taxonomic ranks (i.e. across species and across clades) may be less fruitful than infraspecific comparisons (i.e. within species), given the amount of genetic divergence within the genus. Major *Symbiodinium* clades diverged between 50 and 60 Ma [13,45]; a divergence time similar to bird [46] and primate families [47]. It is thus not surprising that contradictory results have been reported on the molecular mechanisms underlying thermotolerance [48,49]. Comparative transcriptomics of sensitive (*Symbiodinium* C3 K) versus thermotolerant (clade D) species (i.e. from separate clades) could not postulate a clear mechanism for thermal acclimation due to the long divergence time between clade C and D (40 Ma according to [45]). Similarly, Rosic *et al*. [50] were unable to see converging molecular patterns of thermotolerance between *S. pilosum* (=type *A2*), *Symbiodinium psygmophilum* (=type *B2*), *Symbiodinium goreaui* (=type *C1*) and *Symbiodinium trenchii* (=type *D1a*), representing species from highly divergent lineages. In this study, we demonstrate that comparative analysis at infraspecific taxonomic ranks (i.e. genotypes within and between closely related species) will be necessary if we want to understand underlying genetic attributes responsible for variation in thermotolerance.

In all clade A species examined, we find that 26°C is closer to the optimal growth temperature than 32°C. Growth rate appears to be much more affected than F_v/F_m by high temperature. The adverse effects of high temperature (above 32°C) on photosynthesis have been extensively characterized for *Symbiodinium* grown in culture [10,13–15,17]. For instance, in a strain of *S. microadriaticum*, photosynthesis impairment occurs at temperatures above 30°C, stopping completely at higher temperatures of 34–36°C [10]. Our results show that for 11 strains comprising five species of *Symbiodinium* clade A, a temperature of 32°C was high enough to distinguish between sensitive and thermotolerant strains. Notably, thermotolerance is not phylogenetically constrained and instead, there are sensitive, intermediate and tolerant populations within species. Although we were able to statistically categorize thermotolerance into these groups, a gradient in thermotolerance can be observed (figure 5). Some strains might fall into the tolerant side of the gradient, such as members of *S. pilosum* and *S. microadriaticum*, while others such as strains from *S. tridacnidorum* fall into the sensitive side of the tolerance gradient. Nevertheless, the thermotolerance of more strains from each species should be evaluated in order to make broader generalizations.

(b) Combined effect of light and temperature in physiological acclimation

Most investigators recognize the importance of considering the role of multiple stressors in order to improve predictions regarding physiological responses to climate change [51]. Acclimation to high temperature in *Symbiodinium* often

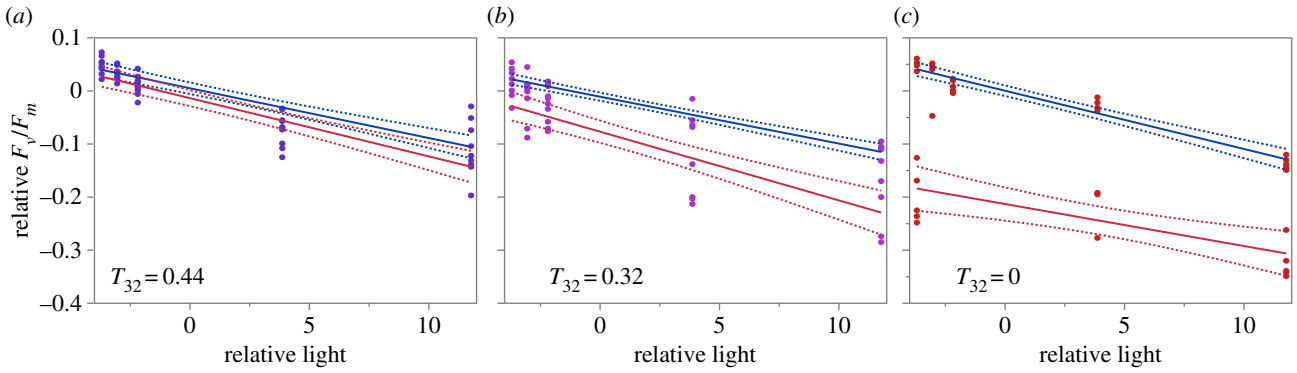

Figure 6. Relative photochemical efficiency of three strains with different thermotolerances under different light conditions. Relative light was calculated by subtracting the total light dose of the experimental conditions (65, 80, 100, 240 and 443 µmol quanta m^2 s^{-1}) from the initial light conditions (100 µmol quanta m^2 s^{-1}). Relative photochemical efficiency was calculated with the initial F_v/F_m minus the value after 12 h at the light/temperature conditions. (*a*) Tolerant strain (*Smic2*), (*b*) intermediate tolerant strain (*Smic1*) and (*c*) sensitive strain (*Stri1*). T_{32}, thermotolerance value. Blue line indicates linear regression of data under control temperature (26°C) and red line indicates linear regression of data high temperature (32°C). Dotted lines indicate confidence intervals.

Table 3. ANCOVA analysis examining the combined effect of temperature and light on *Symbiodinium* in photochemical efficiency (F_v/F_m). F_v/F_m was measured after 12 h of exposure to light/temperature treatments with six technical replicates. Cultures were grown under control (26°C), and high temperature (32°C), and five light intensities 65, 80, 100, 240, 423 µmol quanta m^{-2} s^{-1}. d.f., degrees of freedom. Whole model test: $F_{9,140} = 52.987$, $p < 0.0001$, $R^2 = 0.773$.

response	source	d.f.	*F* ratio	Prob > *F*
photochemical efficiency	strain	2	67.85	less than 0.0001
	temperature	1	138.118	less than 0.0001
	relative light	1	49.565	less than 0.0001
	strain × temperature	2	66.3663	less than 0.0001
	strain × relative light	2	8.1871	0.0004
	temp × relative light	1	4.3963	0.0378

comes with physiological trade-offs, including decreased photochemical activity and reduced growth [52]. It has been observed that during coral bleaching, high temperatures are usually interacting with high levels of light [4] and variation in available nutrients [53]. Combinations of various environmental factors will elicit different physiological responses by different genotypes and species. Having the ability to acclimate to multiple stressors may come with a cost to the organism's physiology. Studying these environmental factors separately and in combination aids the identification of possible trade-offs and any consequences for the functional performance of a particular host–symbiont combination. Our results showed that some tolerant strains had the ability to acclimate well to both high light and high temperature. The strain with intermediate tolerance tested here was affected by high temperature during photoacclimation. By contrast, thermal stress in the sensitive strain *Stri1* was compounded by exposure to high light. Thus, the combined effect of light and temperature exacerbates stress, particularly in thermosensitive species and genotypes.

The ability to acclimate to multiple interacting factors including temperature and light should be further studied, especially among closely related *Symbiodinium* lineages. These physiological attributes will likely determine the future ecological success of certain *Symbiodinium* taxa to changing climatic conditions [54]. Understanding further the synergistic effects of temperature and light on *Symbiodinium* physiology is crucial for predicting the response of coral–algal symbioses to climate change.

5. Conclusion

A comparative approach—examining closely related species and individual genotypes within species—revealed broad physiological diversity among and within *Symbiodinium* spp. Thermotolerance is not species- or clade-specific, but rather widespread and varied among members of the genus. Moreover, the further delineation of species within the genus should improve research on mechanisms of thermotolerance, especially when comparing the performance of certain host–symbiont combinations under various environmental conditions.

Data accessibility. Data are available from the Dryad Digital Repository at: https://doi.org/10.5061/dryad.429vh [55].

Competing interests. We declare we have no competing interests.

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References

1. MacFarling Meure C, Etheridge D, Trudinger C, Steele P, Langenfelds R, van Ommen T, Smith A, Elkins J. 2006 Law Dome CO_2, CH_4 and N_2O ice core records extended to 2000 years BP. *Geophys. Res. Lett.* **33**, L14810. (doi:10.1029/2006GL026152)
2. Thompson LG, Mosley-Thompson E, Brecher H, Davis M, León B, Les D, Lin P-N, Mashiotta T, Mountain K. 2006 Abrupt tropical climate change: past and present. *Proc. Natl Acad. Sci. USA* **103**, 10 536–10 543. (doi:10.1073/pnas.0603900103)
3. Rhein M *et al.* 2013 Chapter 3: Observations: ocean. In *Climate change 2013: the physical science basis*. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (eds TF Stocker *et al.*), pp. 255–316. Cambridge, UK: Cambridge University Press. (doi:10.1017/CBO9781107415324.010)
4. Hoegh-Guldberg O *et al.* 2007 Coral reefs under rapid climate change and ocean acidification. *Science* **318**, 1737–1742. (doi:10.1126/science.1152509)
5. Eakin CM *et al.* 2010 Caribbean corals in crisis: record thermal stress, bleaching, and mortality in 2005. *PLoS ONE* **5**, e13969. (doi:10.1371/journal.pone.0013969)
6. Loya Y, Sakai K, Yamazato K, Nakano Y, Sambali H, van Woesik R. 2001 Coral bleaching: the winners and the losers. *Ecol. Lett.* **4**, 122–131. (doi:10.1046/j.1461-0248.2001.00203.x)
7. Ulstrup KE, Berkelmans R, Ralph PJ, Van Oppen MJ. 2006 Variation in bleaching sensitivity of two coral species across a latitudinal gradient on the Great Barrier Reef: the role of zooxanthellae. *Mar. Ecol. Prog. Ser.* **314**, 135–148. (doi:10.3354/meps314135)
8. Penin L, Adjeroud M, Schrimm M, Lenihan HS. 2007 High spatial variability in coral bleaching around Moorea (French Polynesia): patterns across locations and water depths. *C. R. Biol.* **330**, 171–181. (doi:10.1016/j.crvi.2006.12.003)
9. LaJeunesse TC *et al.* 2010 Host–symbiont recombination versus natural selection in the response of coral–dinoflagellate symbioses to environmental disturbance. *Proc. R. Soc. B* **277**, 2925–2934. (doi:10.1098/rspb.2010.0385)
10. Iglesias-Prieto R, Matta JL, Robins WA, Trench RK. 1992 Photosynthetic response to elevated temperature in the symbiotic dinoflagellate *Symbiodinium microadriaticum* in culture. *Proc. Natl Acad. Sci. USA* **89**, 10 302–10 305. (doi:10.1073/pnas.89.21.10302)
11. Iglesias-Prieto R, Trench RK. 1994 Acclimation and adaptation to irradiance in symbiotic dinoflagellates. I. Responses of the photosynthetic unit to changes in photon flux density. *Mar. Ecol. Prog. Ser.* **113**, 163–175. (doi:10.3354/meps113163)
12. Iglesias-Prieto R, Trench RK. 1997 Acclimation and adaptation to irradiance in symbiotic dinoflagellates. II. Response of chlorophyll–protein complexes to different photon-flux densities. *Mar. Biol.* **130**, 23–33. (doi:10.1007/s002270050221)
13. Tchernov D, Gorbunov MY, de Vargas C, Yadav SN, Milligan AJ, Häggblom M, Falkowski PG. 2004 Membrane lipids of symbiotic algae are diagnostic of sensitivity to thermal bleaching in corals. *Proc. Natl Acad. Sci. USA* **101**, 13 531–13 535. (doi:10.1073/pnas.0402907101)
14. Robison JD, Warner ME. 2006 Differential impacts of photoacclimation and thermal stress on the photobiology of four different phylotypes of *Symbiodinium* (Pyrrhophyta). *J. Phycol.* **42**, 568–579. (doi:10.1111/j.1529-8817.2006.00232.x)
15. Takahashi S, Whitney S, Itoh S, Maruyama T, Badger M. 2008 Heat stress causes inhibition of the de novo synthesis of antenna proteins and photobleaching in cultured *Symbiodinium*. *Proc. Natl Acad. Sci. USA* **105**, 4203–4208. (doi:10.1073/pnas.0708554105)
16. Díaz-Almeyda E, Thomé PE, El Hafidi M, Iglesias-Prieto R. 2011 Differential stability of photosynthetic membranes and fatty acid composition at elevated temperature in *Symbiodinium*. *Coral Reefs* **30**, 217–225. (doi:10.1007/s00338-010-0691-5)
17. Takahashi S, Yoshioka-Nishimura M, Nanba D, Badger MR. 2013 Thermal acclimation of the symbiotic alga *Symbiodinium* spp. alleviates photobleaching under heat stress. *Plant Physiol.* **161**, 477–485. (doi:10.1104/pp.112.207480)
18. Hennige SJ, Suggett DJ, Warner ME, McDougall KE, Smith DJ. 2009 Photobiology of *Symbiodinium* revisited: bio-physical and bio-optical signatures. *Coral Reefs* **28**, 179–195. (doi:10.1007/s00338-008-0444-x)
19. Coffroth MA, Santos SR. 2005 Genetic diversity of symbiotic dinoflagellates in the genus *Symbiodinium*. *Protist* **156**, 19–34. (doi:10.1016/j.protis.2005.02.004)
20. Pochon X, Gates RD. 2010 A new *Symbiodinium* clade (Dinophyceae) from soritid foraminifera in Hawai'i. *Mol. Phylogenet. Evol.* **56**, 492–497. (doi:10.1016/j.ympev.2010.03.040)
21. LaJeunesse TC, Lee SY, Gil-Agudelo DL, Knowlton N, Jeong HJ. 2015 *Symbiodinium necroappetens* sp. nov. (Dinophyceae): an opportunist 'zooxanthella' found in bleached and diseased tissues of Caribbean reef corals. *Eur. J. Phycol.* **50**, 223–238. (doi:10.1080/09670262.2015.1025857)
22. Lee SY, Jeong HJ, Kang NS, Jang TY, Jang SH, Lajeunesse TC. 2015 *Symbiodinium tridacnidorum* sp. nov., a dinoflagellate common to Indo-Pacific giant clams, and a revised morphological description of *Symbiodinium microadriaticum* Freudenthal, emended Trench & Blank. *Eur. J. Phycol.* **50**, 155–172. (doi:10.1080/09670262.2015.1018336)
23. Parkinson JE, Coffroth MA, LaJeunesse TC. 2015 New species of clade B *Symbiodinium* (Dinophyceae) from the greater Caribbean belong to different functional guilds: *S. aenigmaticum* sp. nov., *S. antillogorgium* sp. nov., *S. endomadracis* sp. nov., and *S. pseudominutum* sp. nov. *J. Phycol.* **51**, 850–858. (doi:10.1111/jpy.12340)
24. Thornhill DJ, Lewis AM, Wham DC, LaJeunesse TC. 2014 Host–specialist lineages dominate the adaptive radiation of reef coral endosymbionts. *Evolution* **68**, 352–367. (doi:10.1111/evo.12270)
25. LaJeunesse T. 2002 Diversity and community structure of symbiotic dinoflagellates from Caribbean coral reefs. *Mar. Biol.* **141**, 387–400. (doi:10.1007/s00227-002-0829-2)
26. Goulet T, Coffroth M. 2003 Genetic composition of zooxanthellae between and within colonies of the octocoral *Plexaura kuna*, based on small subunit rDNA and multilocus DNA fingerprinting. *Mar. Biol.* **142**, 233–239. (doi:10.1007/s00227-002-0936-0)
27. Goulet TL, Coffroth MA. 2003 Stability of an octocoral–algal symbiosis over time and space. *Mar. Ecol. Prog. Ser.* **250**, 117–124. (doi:10.3354/meps250117)
28. Finney JC, Pettay DT, Sampayo EM, Warner ME, Oxenford HA, LaJeunesse TC. 2010 The relative significance of host–habitat, depth, and geography on the ecology, endemism, and speciation of coral endosymbionts in the genus *Symbiodinium*. *Microb. Ecol.* **60**, 250–263. (doi:10.1007/s00248-010-9681-y)
29. Prada C, McIlroy SE, Beltrán DM, Valint DJ, Ford SA, Hellberg ME, Coffroth MA. 2014 Cryptic diversity hides host and habitat specialization in a gorgonian–algal symbiosis. *Mol. Ecol.* **23**, 3330–3340. (doi:10.1111/mec.12808)
30. Berkelmans R, van Oppen MJ. 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. R. Soc. B.* **273**, 2305–2312. (doi:10.1098/rspb.2006.3567)
31. Warner ME, LaJeunesse TC, Robison JD, Thur RM. 2006 The ecological distribution and comparative photobiology of symbiotic dinoflagellates from reef corals in Belize: potential implications for coral bleaching. *Limnol. Oceanogr.* **51**, 1887–1897. (doi:10.4319/lo.2006.51.4.1887)
32. LaJeunesse TC, Pettay DT, Sampayo EM, Phongsuwan N, Brown B, Obura DO, Hoegh-Guldberg O, Fitt WK. 2010 Long-standing environmental conditions, geographic isolation and host–symbiont specificity influence the relative ecological dominance and genetic diversification of coral endosymbionts in the genus *Symbiodinium*. *J. Biogeogr.* **37**, 785–800. (doi:10.1111/j.1365-2699.2010.02273.x)
33. 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. (doi:10.1038/srep08562)
34. Glynn PW, Maté JL, Baker AC, Calderón MO. 2001 Coral bleaching and mortality in Panama and Ecuador during the 1997–1998 El Niño–Southern Oscillation event: spatial/temporal patterns and comparisons with the 1982–1983 event. *Bull. Mar. Sci.* **69**, 79–109.

35. Rowan R. 2004 Coral bleaching: thermal adaptation in reef coral symbionts. *Nature* **430**, 742. (doi:10.1038/430742a)
36. Wham DC, Ning G, LaJeunesse TC. 2017 *Symbiodinium glynnii* sp. nov., a species of stress-tolerant symbiotic dinoflagellates from pocilloporid and montiporid corals in the Pacific Ocean. *Phycologia* **56**, 396–409. (doi:10.2216/16-86.1)
37. McGinley MP, Aschaffenburg MD, Pettay DT, Smith RT, LaJeunesse TC, Warner ME. 2012 Transcriptional response of two core photosystem genes in *Symbiodinium* spp. exposed to thermal stress. *PLoS ONE* **7**, e50439. (doi:10.1371/journal.pone.0050439)
38. Sawall Y, Al-Sofyani A, Hohn S, Banguera-Hinestroza E, Voolstra CR, Wahl M. 2015 Extensive phenotypic plasticity of a Red Sea coral over a strong latitudinal temperature gradient suggests limited acclimatization potential to warming. *Sci. Rep.* **5**, 8940. (doi:10.1038/srep08940)
39. Blank RJ. 1987 Cell architecture of the dinoflagellate *Symbiodinium* sp. inhabiting the Hawaiian stony coral *Montipora verrucosa*. *Mar. Biol.* **94**, 143–155. (doi:10.1007/BF00392906)
40. LaJeunesse TC. 2001 Investigating the biodiversity, ecology, and phylogeny of endosymbiotic dinoflagellates in the genus *Symbiodinium* using the ITS region: in search of a 'species' level marker. *J. Phycol.* **37**, 866–880. (doi:10.1046/j.1529-8817.2001.01031.x)
41. Kirchman D, Ducklow H, Mitchell R. 1982 Estimates of bacterial growth from changes in uptake rates and biomass. *Appl. Environ. Microbiol.* **44**, 1296–1307.
42. Ellison GN, Gotelli NJ. 2004 *A primer of ecological statistics*. Sunderland, MA: Sinauer.
43. Dunne RP. 2010 Synergy or antagonism—interactions between stressors on coral reefs. *Coral Reefs* **29**, 145–152. (doi:10.1007/s00338-009-0569-6)
44. Parkinson JE, Baums IB. 2014 The extended phenotypes of marine symbioses: ecological and evolutionary consequences of intraspecific genetic diversity in coral–algal associations. *Front. Microbiol.* **5**, 445. (doi:10.3389/fmicb.2014.00445)
45. Pochon X, Montoya-Burgos JI, Stadelmann B, Pawlowski J. 2006 Molecular phylogeny, evolutionary rates, and divergence timing of the symbiotic dinoflagellate genus *Symbiodinium*. *Mol. Phylogenet. Evol.* **38**, 20–30. (doi:10.1016/j.ympev.2005.04.028)
46. Härlid A, Janke A, Arnason U. 1997 The mtDNA sequence of the ostrich and the divergence between paleognathous and neognathous birds. *Mol. Biol. Evol.* **14**, 754–761. (doi:10.1093/oxfordjournals.molbev.a025815)
47. Steiper ME, Young NM. 2006 Primate molecular divergence dates. *Mol. Phylogenet. Evol.* **41**, 384–394. (doi:10.1016/j.ympev.2006.05.021)
48. Ladner JT, Barshis DJ, Palumbi SR. 2012 Protein evolution in two co-occurring types of *Symbiodinium*: an exploration into the genetic basis of thermal tolerance in *Symbiodinium* clade D. *BMC Evol. Biol.* **12**, 217. (doi:10.1186/1471-2148-12-217)
49. Barshis DJ, Ladner JT, Oliver TA, Palumbi SR. 2014 Lineage-specific transcriptional profiles of *Symbiodinium* spp. Unaltered by heat stress in a coral host. *Mol. Biol. Evol.* **31**, 1343–1352. (doi:10.1093/molbev/msu107)
50. Rosic N, Kaniewska P, Chan, C.-K.K, Ling EYS, Edwards D, Dove S, Hoegh-Guldberg O. 2014 Early transcriptional changes in the reef-building coral *Acropora aspera* in response to thermal and nutrient stress. *BMC Genomics* **15**, 1052. (doi:10.1186/1471-2164-15-1052)
51. Todgham AE, Stillman JH. 2013 Physiological responses to shifts in multiple environmental stressors: relevance in a changing world. *Integr. Comp. Biol.* **53**, 539–544. (doi:10.1093/icb/ict086)
52. Cunning R, Silverstein RN, Baker AC. 2015 Investigating the causes and consequences of symbiont shuffling in a multi-partner reef coral symbiosis under environmental change. *Proc. R. Soc. B* **282**, 20141725. (doi:10.1098/rspb.2014.1725)
53. Coles SL, Brown BE. 2003 Coral bleaching—capacity for acclimatization and adaptation. *Adv. Mar. Biol.* **46**, 183–223. (doi:10.1016/S0065-2881(03)46004-5)
54. Chevin L-M, Lande R, Mace GM. 2010 Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. *PLoS Biol.* **8**, e1000357. (doi:10.1371/journal.pbio.1000357)
55. Díaz-Almeyda EM, Prada C, Ohdera AH, Moran H, Civitello DJ, Iglesias-Prieto R, Carlo TA, LaJeunesse TC, Medina M. 2017 Data from: Intraspecific and interspecific variation in thermotolerance and photoacclimation in *Symbiodinium* dinoflagellates. Dryad Digital Repository. (http://dx.doi.org/10.5061/dryad.429vh)