**Detailed Methods and additional Figures**

**Experimental corals, *Symbiodinium* inoculation, and genetic identification**

Three independent heat stress experiments were carried out using *Acropora tenuis* juveniles raised after the spawning events of 2003-2005 at Magnetic Island (19°.10'S, 146°.50'E) in the central section of the Great Barrier Reef. The pilot study and first experiment used six-month old juveniles, while those in the second experiment were 16 months old. Type C1 or D symbionts were isolated from adult *A. tenuis* and *A. millepora* colonies, respectively, and used to inoculate aposymbiotic coral juveniles, which had been settled on terra-cotta tiles and maintained in 1μm filtered seawater as described in Little et al. (2004). Following uptake of *Symbiodium,* tiles with attached juveniles were suspended vertically on rods, which were then hung between star pickets to grow on the reef. Complete ITS1 sequences of D symbionts isolated from *A. millepora* (GenBank accession number EU024793) were identical to those found in *A. tenuis* juveniles naturally infected at this location (Little et al. 2004). Requirements for large quantities of D symbionts during inoculations precluded the use of naturally occurring *A. tenuis* juveniles as a source of symbiont cells, however, D symbionts from adult *A. millepora* colonies were readily taken up by the juveniles in this study. Furthermore, a recent survey of 55 taxa at the same study site including hard and soft corals revealed that at least 21 of these taxa had symbionts with identical sequences to both the D symbionts used to inoculate juveniles in this study and the D symbionts taken up naturally by recruits in the reef (D. Abrego, unpublished data).

Genetic verification of *Symbiodinium* type hosted by the juvenile corals took place after initial inoculation and again immediately before the start of each experiment. This

was performed using Single Stranded Conformation Polymorphism (SSCP) of the ITS1 region of the nuclear rDNA. For the pilot study, 6 colonies per association were randomly selected for verification of symbiont identity and all had only the *Symbiodinium* type initially offered, within the limit of detection of the SSCP technique (5-10% of relative abundance; Fabricius et al. 2004).. Additionally, 8 colonies per clade were randomly selected at the end of this study to verify that surviving colonies retained only the *Symbiodinium* type offered. For the first experiment, 20 colonies were randomly selected per association and in all but two juveniles, the presence of only the symbiont initially offered was confirmed. The two exceptions were from D-juveniles, where type C1 was detected in approximately equal proportions. Because the coral colonies in the second experiment were larger compared to those in the previous experiments, a small piece of every experimental colony used was checked prior to experimentation and confirmed to harbor only the *Symbiodinium* type initially offered.

**Experimental design**

For the pilot study and the first full scale experiment, four temperature treatments (28°C, 30°C, 31°C, 32°C) were selected ranging from ambient, non-stress conditions to the temperature at which bleaching occurs on local reefs. For the second full scale experiment, only three temperatures were selected (26°C, 29°C, 32°C) due to the reduced number of colonies available for replication (n=45 C1-corals; n=26 D-corals). In the latter experiment, a control temperature of 26°C was selected to match ambient, winter water temperatures at the field site where corals were kept. For each experiment, C1 and D-corals were divided among three replicate tanks per temperature treatment. The number of replicate colonies per temperature treatment ranged from 26-370 depending on

the experiment (See Suppl. Table 1 for a summary of assays, experimental conditions, and sample size for each experiment).

Irradiance levels were selected based on the range of intensities (120-320 µmol photons $m^{-2}$ $s^{-1}$) experienced by juveniles *in situ* at the grow-out site. Intensities were at the low end of the range in the pilot study to avoid light stress (130 µmol photons $m^{-2}$ $s^{-1}$). To test the impact of light dose, the photoperiod was initially 7.5 hours per day and then raised to 10 hours per day for the second half of the experiment. The increased number of bleached juveniles after the photoperiod was lengthened provided evidence for the importance of light in determining the bleaching response of corals. Hence, two light treatments were used in the first experiment, corresponding to the low (160 µmol photons $m^{-2}$ $s^{-1}$) and high (360 µmol photons $m^{-2}$ $s^{-1}$) ends of the *in situ* range. For the second experiment, irradiance intensity for the single light treatment was selected to approximate the middle of the *in situ* range (250 umol photons $m^{-2}$ $s^{-1}$). Throughout both the first and second experiments, the photo-period was maintained at 10hrs light: 14hrs dark.

**Experimental setup**

In preparation for all three experiments, tiles with attached juveniles were retrieved from the reef and macro-algae and other organisms were removed before being assigned haphazardly to experimental tanks. Due to differences in initial rates of settlement and survival of corals whilst on the reef, the number of colonies per tile varied but there was approximately the same number of C1 and D colonies per temperature treatment (Suppl. Table 1).

To ensure accurate temperature control in experimental treatments, filtered seawater (0.5 µm) was pumped into 300-500 L reservoirs in a temperature-controlled room. Each

reservoir was fitted with a single 1 or 2 kW titanium heater with digital controllers that switched on/off if the water temperature varied by more than ± 0.1°C from target temperatures. From each reservoir, the water was pumped at 3 L/min into three replicate tanks (approx. 60 L each) fitted with a small power head pump to maintain water movement, and an air stone that provided a small stream of bubbles. Water temperature in the tanks was monitored by thermistors connected to data loggers (Innotech Genesis II) that recorded temperatures every 15 seconds. Target temperatures never varied by more than ± 0.3°C throughout each experiment (see Suppl. Table 1 for summary of experimental setups for all three experiments). Metal halide lights provided the appropriate spectral output for photosynthesis (250W, 20K, Sylvania). In every experiment coral juveniles were allowed to acclimate to experimental light levels for at least five days before the start of heating. This period was sufficient for maximum quantum yield values to stabilize. Temperatures were increased slowly (0.3°C/4hrs) from a staggered start so that target temperatures in each treatment were reached at the same time.

**$O_2$ microelectrode characterization of photosynthesis and respiration**

In order to measure rates of gross and net photosynthesis as well as respiration, we used an oxygen microelectrode connected to a pico-amperemeter (PA2000, Unisense A/S, Denmark) which recorded measuring signals on a strip chart recorder (Kipp & Zonen, The Netherlands). Each colony was placed in a custom-built flow chamber (25 × 10 × 10 cm) through which seawater at experimental temperatures ± 0.5ºC was circulated at a flow velocity of ~1 cm $s^{-1}$. An adjustable fibre-optic light source (Schott KL-2500) with a 250 W halogen lamp, fitted with a collimating lens and calibrated against a

quantum irradiance meter (LiCor 192),was used for homogeneous, vertical illumination of the coral samples. The maximum irradiance level matched the level in the heat experiment (250 µmol photons $m^{-2}$ $s^{-1}$). To minimize heterogeneity in the light climate caused by complexity in the skeletal structures of corals, a fully light exposed coral surface was selected for positioning of the microelectrode. A manual micromanipulator (MM33, Märtzhäuser, Germany) enabled us to place the microelectrode in direct contact with the coral tissue whilst observing the samples with a dissecting microscope. Linear calibration of the electrode was performed at experimental temperatures by recording signals in air-saturated seawater and $O_2$-free seawater, respectively. The $O_2$ concentration of air-saturated seawater at experimental temperatures and salinity (35 ppm) was obtained from tabulated values (www.unisense.com).

The light-dark shift technique is based on the assumption that the immediate (<1 second) oxygen depletion following the eclipse of the light source is equal to the photosynthetic oxygen production during the previous light period and thus requires physiological steady-state (obtained in this case after 10 minutes at 250 µmol photons $m^{-2}$ $s^{-1}$) (Kühl et al. 1996; Revsbech & Jorgensen 1983). In order to obtain accurate Pg measurements, the oxygen microelectrode must be small (< 100 µm) and exhibit low stirring sensitivity and fast response time (Revsbech 1989).

**Chlorophyll *a* content and xanthophyll pigments**

The HPLC system comprised: In-Line vacuum degasser, controller and quaternary pump (Waters™, 600), autosampler (Waters™, 717plus) and a photodiode array detector (PDA, Waters™, 996). System control, data collection and integration were performed using the software Empower Pro (Waters Corporation, 2002). The method was adapted

from van Heukelem and Thomas (2001). Coral fragments were disrupted with an ultrasonic probe for 1 minute before and after extraction. The extract was then centrifuged at 15,000 rpm for 2 minutes and the clarified supernatant extract was mixed 1:1 (v/v) in solvent A (see below), before injection (10 µl) into a Gemini C18 column (3 µm particle size, 110Å pore, 50 x 4.6 mm, Phenomenex®) at room temperature. The coral skeletons were kept for surface area calculations. Pigments were separated using a binary mobile phase system (see Suppl. Table 2 for Analytical Gradient Protocol). All the organic solvents used were HPLC-grade. Solvent A was prepared by diluting 2.8 mL 1.0M tetra butyl ammonium acetate (TBAA, Sigma-Aldrich) into 90 mL Milli-Q water. The pH was adjusted to 6.5 and the mixture was diluted to 100 mL to give 28 mM TBAA. Ninety mL of this solution was then diluted with 210 mL of methanol and filtered under vacuum through Millipore organic solvent filters. Solvent B was prepared by mixing 1:1 (v/v) methanol:acetone and filtered under vacuum as above.

The area of the peaks corresponding to chl *a* (retention time 11.74 ±0.02 min), diadinoxanthin (retention time 9.26 ±0.02 min), and diatoxanthin (retention time 9.70 ±0.02 min) were obtained using the Empower Pro software. Manual correction of peak baselines was performed if necessary to make sure that no adjacent peaks/shoulders were used in the calculation of peak areas. Pigment concentrations were calculated using relative response factors for each pigment standard. Pigment amounts were standardized to the surface area of the fragment from which they were extracted.

**References**

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**Suppl. Table 1**. Physiological assay, experimental setup and number of colonies for each heat stress experiment. HL: High light, LL: Low light.

Physiological assay for each experiment

| Experiment | Assay |
|---|---|
| Pilot | Bleaching condition, photochemical efficiency |
| 1 | Bleaching condition, photochemical efficiency |
| 2 | Photochemical efficiency, pressure over PSII, $O_2$ production and consumption, pigment quantification and absorption efficiency |

Experimental conditions for each heat stress experiment

| Experiment | Temperature treatments (ºC) | Light intensity (µmol photons $m^{-2}$ $s^{-1}$) | Length of experiment (days) |
|---|---|---|---|
| Pilot | 28, 30, 31, 32 | 128 ± 20 | 31 |
| 1 | 28, 30, 31, 32 | HL: 362 ± 9<br>LL: 158 ± 8 | 17 |
| 2 | 26, 29, 32 | 240 ± 15 | 18 |

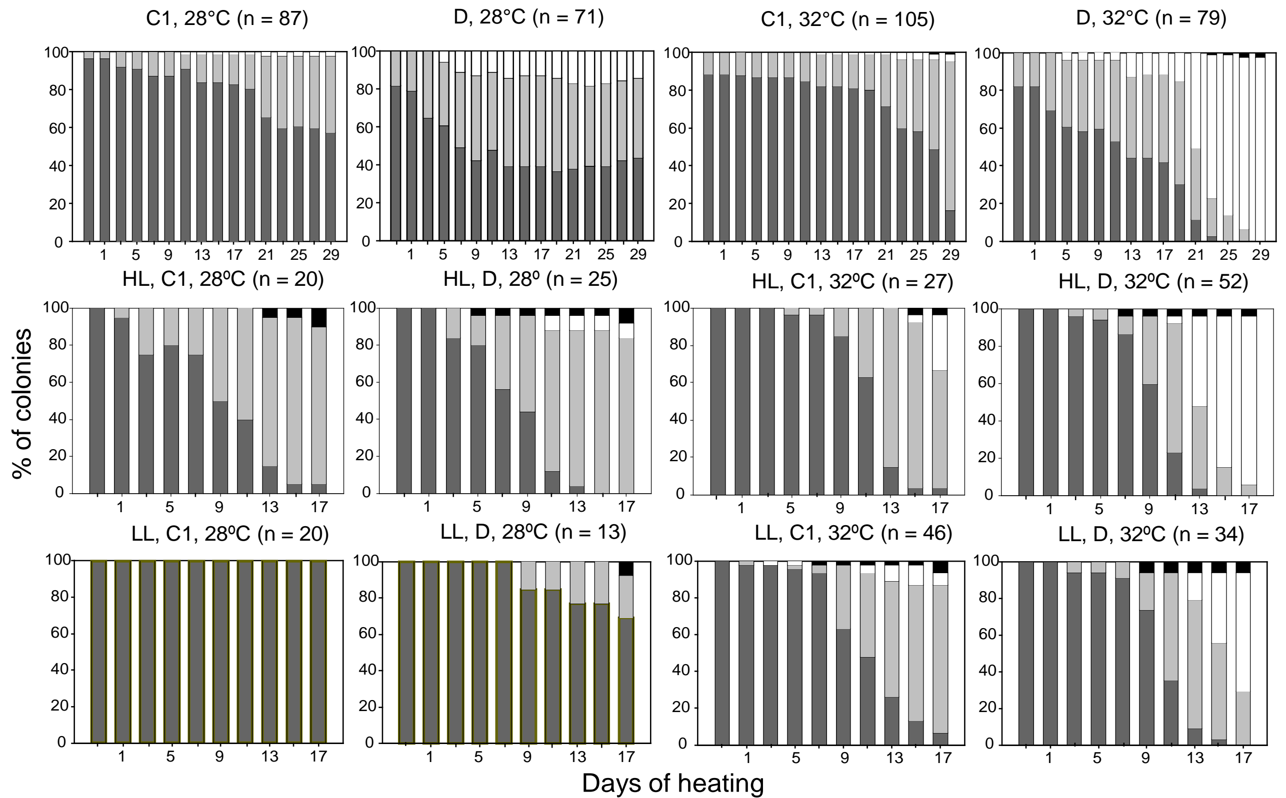
Sample size (n) used in each experiment

| Exp | n per type | | Range in sample size (n) per *Symbiodinium* type for each temp. | | n for fluorescence measurements |
|---|---|---|---|---|---|
| | C1 | D | C1 | D | |
| Pilot | 370 | 324 | 77-105 | 71-86 | 24 |
| 1 HL | 98 | 121 | 18-33 | 16-52 | 10-17 |
| 1 LL | 137 | 97 | 20-46 | 13-34 | 10-17 |
| 2 | 45 | 26 | 13-16 | 7-10 | 7-13 |

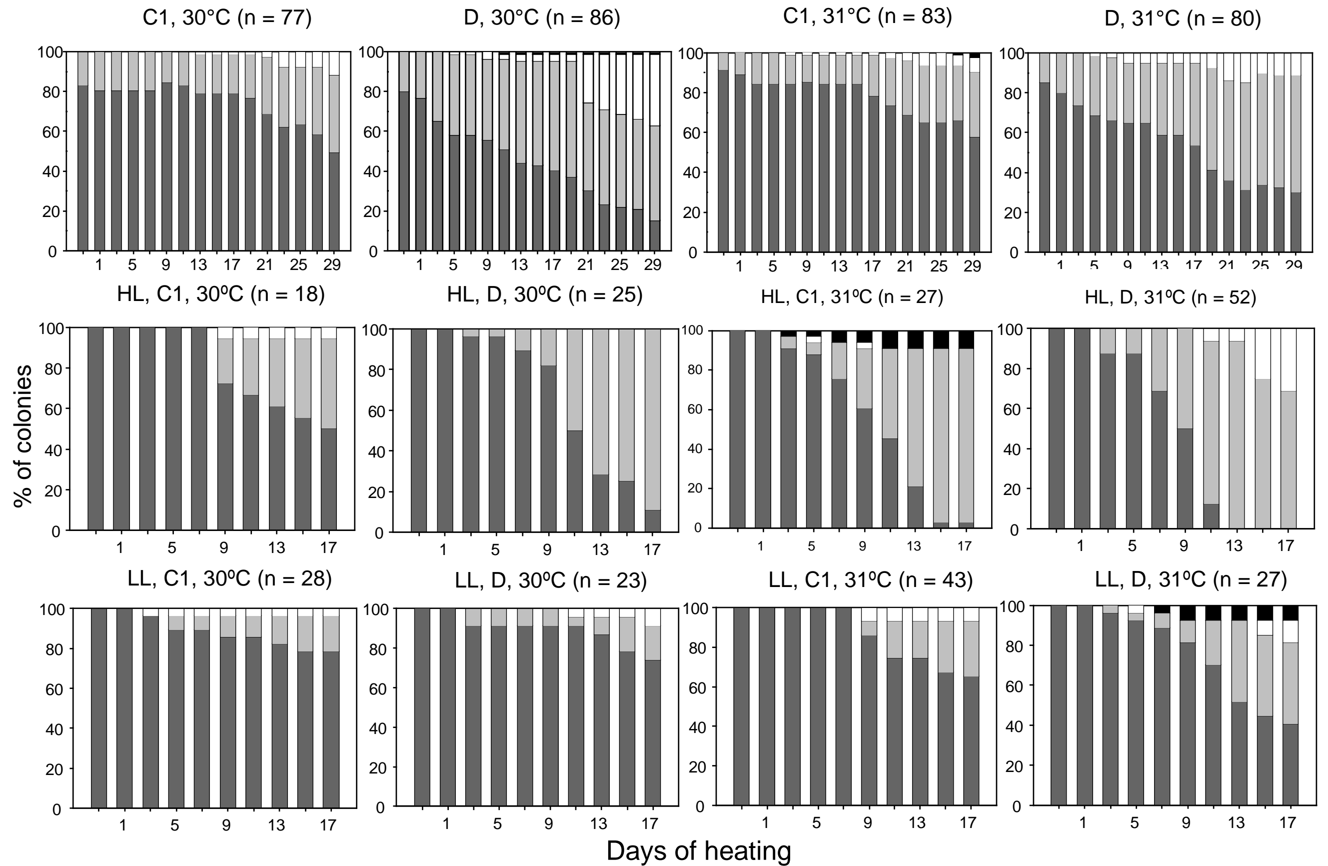
**Suppl. Table 2.** HPLC analytical gradient protocol. Flow rate was maintained at 1 ml $min^{-1}$ for the duration of the analysis.

| Time (min) | Solvent A % [70:30(v/v) methanol:28mM aqueous tetrabutyl ammonium acetate (TBAA)] | Solvent B % [50:50(v/v) acetone:MeOH] | Condition |
|---|---|---|---|
| 0 | 75 | 25 | Injection |
| 5 | 0 | 100 | Linear gradient |
| 10 | 0 | 100 | Linear gradient |
| 11 | 75 | 25 | Linear gradient |
| 18 | 75 | 25 | Equilibration |

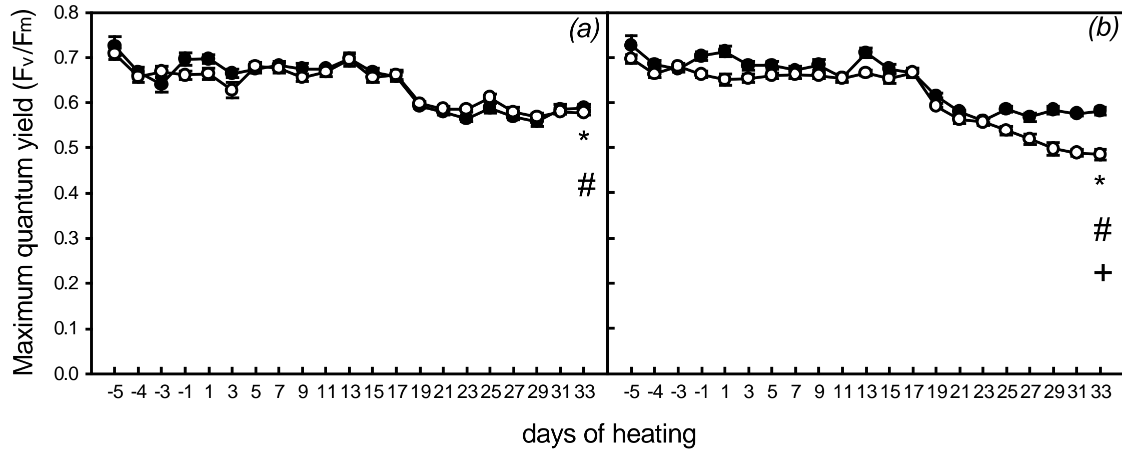
**Suppl. Fig. 1a**. Visual scoring of colonies hosting *Symbiodinium* type C1 or D. Top row shows results for the pilot study and bottom rows are from experiment 1. HL denotes high light treatment (362 μmol photons $m^{-2}$ $s^{-1}$), LL denotes low light treatment (158 μmol photons $m^{-2}$ $s^{-1}$). Four categories were used to quantify bleaching condition and mortality (normal, pale, bleached, dead). *Symbiodinium* type, temperature treatment (28ºC or 32ºC) and sample size (n) are shown for each graph.



**Suppl. Fig. 1b**. Visual scoring of colonies hosting *Symbiodinium* type C1 or D. Top row shows results for pilot study and bottom rows are from experiment 1. HL denotes high light treatment (362 µmol photons $m^{-2}$ $s^{-1}$), LL denotes low light treatment (158 µmol photons $m^{-2}$ $s^{-1}$). Four categories were used to quantify bleaching condition and mortality ( normal, pale, bleached, dead). *Symbiodinium* type, temperature treatment (30ºC or 31ºC) and sample size (n) are shown for each graph.



**Suppl. Fig. 2a-b**. Maximum quantum yield ($F_v/F_m$) of corals hosting either *Symbiodinium* C1 (●) or D (○) at 30 (a) and 31°C (b). Values are means ± SE for each *Symbiodinium* clade (n=24). * and # notations refer to significant differences over time within C1 and D-corals respectively. + denotes a difference between C1 and D-corals. Comparisons are by Mann-Whitney U test.



**Suppl. Fig. 3a-h**. Maximum quantum yield ($F_v/F_m$) of corals hosting either *Symbiodinium* C1 (●) or D (○). Graphs on the left side (a-d) are for corals in the high light treatment (362 µmol photons $m^{-2}$ $s^{-1}$). Graphs on the right side (e-h) are for corals in the low light treatment (158 µmol photons $m^{-2}$ $s^{-1}$).Values are means ± SE for each *Symbiodinium* clade (n=10-17). * and # notations refer to significant differences over time within C1 and D-corals respectively. + denotes a difference between C1 and D-corals. Comparisons are by Mann-Whitney U test.