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Response of two species of Indo-Pacific corals, *Porites cylindrica* and *Stylophora pistillata*, to short-term thermal stress: The host does matter in determining the tolerance of corals to bleaching

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ABSTRACT

The role of both host and dinoflagellate symbionts was investigated in the response of reef-building corals to thermal stress in the light. Replicate coral nubbins of *Stylophora pistillata* and *Porites cylindrica* from the GBR were exposed to either 28 °C (control) or 32 °C for 5 days before being returned to an ambient reef temperature (28 °C). *S. pistillata* was found to contain either *Symbiodinium* genotype C1 or C8a, while *P. cylindrica* had type C15 based on ITS genotyping. Analysis of the quantum yield of photosystem (PS) II fluorescence of the symbionts in *P. cylindrica* showed that light-induced excitation pressure on the C15 *Symbiodinium* was significantly less, and the steady state quantum yield of PSII fluorescence at noon ($\Delta F/Fm'$) greater, than that measured in C1/C8a *Symbiodinium* sp. from *S. pistillata*. Immunoblots of the PS II D1 protein were significantly lower in *Symbiodinium* from *S. pistillata* compared to those in *P. cylindrica* after exposure to thermal stress. The biochemical markers, heat-stress protein (HSP) 70 and superoxide dismutase (SOD), were significantly greater in *P. cylindrica* before the experiment, and both species of coral increased their biosynthesis of HSP 70 and SOD when exposed to thermal stress. Concentrations of MAAs, glycerol, and lipids were not significantly affected by thermal stress in these experiments, but DNA damage was greater in heat-stressed *S. pistillata* compared to *P. cylindrica*. There was minimal coral mucus, which accounts for up to half of the total energy budget of a coral and provides the first layer of defense for invading microbes, produced by *S. pistillata* after heat stress compared to *P. cylindrica*. It is concluded that *P. cylindrica* contains a heat resistant C15 *Symbiodinium* and critical host proteins are present at higher concentrations than observed for *S. pistillata*, the combination of which provides greater protection from bleaching conditions of high temperature in the light.

1. Introduction

Some species of Scleractinian corals are more resistant than others to environmental stresses that result in the loss of their symbiotic dinoflagellate symbionts (=zooxanthellae) or algal pigments (=bleaching), such as observed during El-Nino Southern Oscillation (ENSO) events when seawater temperatures exceed their warm-seasonal means (Hoegh-Guldberg, 1999; Lesser, 2004). While bleached corals lose most of their symbiotic dinoflagellates and appear white after relatively short high-temperature exposures, adjacent colonies of the same, or another, species may display normal coloration for weeks or even months living in the same conditions on the same reef (Edmunds, 1994; Hoegh-Guldberg and Salvat, 1995; Marshall and Baird, 2000). The primary basis for these differences in tolerance is still largely unknown and likely involves the synergistic effects of thermal stress with other factors such as solar radiation (Fitt

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et al., 2001; Lesser and Farrell, 2004), and physiological differences between corals and their symbionts.

Several factors can apparently determine whether a coral colony loses enough symbiotic algae to enable the white skeleton to show through the live tissue and thus be visually "bleached". One such characteristic is tissue thickness (Hoegh-Guldberg, 1999). Corals from genera such as *Porites* that have thicker tissues and appear more robust to thermal stress than corals from genera such as *Acropora* which has thinner tissues (Hoegh-Guldberg and Salvat, 1995; Loya et al., 2001). Thermal stress and tissue thickness also interact as shown by Glynn and D'Croz (1990) and Fitt et al. (2000), who documented steady decreases in tissue biomass and symbiont density during summer months, and interpreted it as an increase in metabolic demand and subsequent use of stored energy reserves.

The type of symbiont found in a particular host, and its ability to tolerate environmental change, has also been thought to exert a major influence on the ability of reef-building corals to survive high-temperature stress. Eight major clades have been identified among the *Symbiodinium* sp. that form symbioses with invertebrates and protozoans (Coffroth and Santos, 2005). Of these, four show a regular association with reef-building corals. Rowan et al. (1997) initially described different clades of *Symbiodinium* occurring sympatrically within individual corals of the genus *Montastraea* in the Caribbean. These clades also appeared to distribute themselves in relationship to environmental variables such as solar radiation, where clades A and B dominated the light exposed tops of coral colonies while clade C occurred primarily on the shaded sides of the colony. Some of the variability in the observed micro- and macrohabitat differences associated with distribution of a species of coral can be related back to particular types of symbionts living in that particular host species. For instance, Iglesias-Prieto et al. (2004) reported significant correlations between the depth distribution of *Pocillopora verrucosa* and *Pavona gigantea* colonies and the ability of their resident symbionts (from different clades) to photosynthesize under different ranges of irradiances, leading to the conclusion that the ability of different symbionts to occupy specific light environments, or niches, is strongly influenced (in extant populations) by the genetically determined physiological capabilities of their algal partners.

The diversity of *Symbiodinium* plus their varying physiological characteristics has led to the hypothesis that the species of *Symbiodinium* is the primary determinant of thermal tolerance of coral-dinoflagellate associations. Several studies have tested the physiological tolerances of *Symbiodinium* and found that there are distinct differences in ability to survive warmer than normal conditions (Fitt and Warner, 1995; Warner et al., 1996; Kinzie et al., 2001; Bhagooli and Hidaka, 2003; Berkelmans and van Oppen, 2006). For example, colonies of the ubiquitous reef coral, *Acropora millepora*, that harbors clade D *Symbiodinium*, exhibits a 1 to 1.5 °C greater thermal tolerance than colonies with clade C *Symbiodinium* (Berkelmans and van Oppen, 2006). Studies like these suffer from the problem that the measurements have concentrated on *Symbiodinium* and have not explored the possibility that other features of the association (i.e. host metabolism) may play an equally important role in determining the thermal tolerance of reef-building corals.

If the host plays a role, then the hypothesis that corals simply shuffle or swap their *Symbiodinium* for clades that are more thermally tolerant (e.g., Buddemeier and Fautin, 1993; Baker, 2001) does not tell the whole story. There are dynamic photoprotective mechanisms in both the host and zooxanthellae that include ultraviolet radiation absorbing mycosporine-like amino acids (MAAs) (Shick and Dunlap, 2002; Lesser, 2004), excess excitation energy dissipation in PSII via the xanthophyll cycle (Brown et al., 1999; Gorbunov et al., 2001), the expression of heat-shock proteins and other stress markers (Black et al., 1995; Downs et al., 2000; Lesser and Farrell, 2004), the up-regulation of antioxidant enzymes (Lesser, 1996; Lesser and Farrell, 2004; Lesser, 2006), host energy reserve utilization (Porter et al., 1989; Grottoli et al., 2004, 2006), and heterotrophic plasticity (Grotolli et al., 2006)—all presumably have underlying influences on any response to thermal stress, and hence, contribute to the overall differences within and between species in regard to their bleaching sensitivity.

The purpose of the current study was to apply biochemical, cellular, and molecular measurements in order to provide a more integrated understanding of the heat-stress response in two ubiquitous Indo-Pacific reef corals: the branching corals that are known to be very susceptible (*Stylophora pistillata*) and resistant (*Porites cylindrica*) to heat stress (e.g. Loya et al., 2001, Visram and Douglas, 2007). We found that these species have different tolerances to thermal stress based on the clade of the *Symbiodinium* (the patterns of fluorescence and the amount of the D1 protein), DNA damage (as assessed by cyclobutane pyrimidine dimers), the tissue biomass (as expressed as protein), and depletion of mucus reserves from *S. pistillata* but not *P. cylindrica*. There was a significant increase in the expression of SOD (16 kDa) and HSP 70 (72 kDa) proteins in heat-stressed *P. cylindrica*, compared to controls and *S. pistillata*, but no differences in MAAs, lipid, or immuoblots of host proteins.

2. Materials and methods

2.1. Experimental design

All experiments and field assessments were conducted at the Heron Island Research Station (23°25′S, 152°07′E), during the austral summer (February–March) of 2002. *S. pistillata* was collected from 2–4 m depth on the outer reef and is infrequently found in the lagoon, whereas *P. cylindrica* was collected from 0–2 m depth from the lagoon, though it is also found on the leeward reef of Heron Island. Replicate fragments (ramets or nubbins) from multiple colonies (genets) were placed in outdoor seawater tables covered with neutral density screening to reduce solar radiation to levels measured in the field around Heron Island at the depth of collection. Nubbins were haphazardly mounted in epoxy filled caps and maintained in these tables for one week prior to experimental manipulation to allow for wound healing. Three seawater tables were designated as the high temperature treatment and heated to 32 ± 1 °C (mean ± SD) while three seawater tables were maintained as controls (28 ± 1 °C). After 5 days of exposure to elevated seawater temperatures the high temperature treatment was returned to control temperatures of 28 ± 1 °C for an additional 5 days. On each sampling day at least three replicate nubbins of each coral species were removed from control and experimental seawater tables and analyzed as described below. There were enough replicates of the corals that three or more pieces were available for use in the assays listed below. The number of coral colonies was impossible to determine, because of the branching nature of both species.

Environmentally relevant solar ultraviolet radiation (UVR; 290–400 nm), including ultraviolet-B (UV-B; 290–320 nm) and ultraviolet-A (UV-A; 320–400 nm), as well as photosynthetically active radiation (PAR: 400–700 nm), were measured using a wavelength and radiometrically calibrated (NIST traceable standards) CCD spectrometer (Ocean Optics S2000, 200–1100 nm, 2048 elements, stray light <0.05% at 600 nm; <0.10% at 435 nm, and a fiber optics cable with immersion effect corrected cosine collector) at noon (±1 h) during the experiments. Three scans were taken and the mean reported in units of W m^{-2} nm^{-1}. Integrated values of PAR and unweighted UVR (W m^{-2}) were measured for the experimental set-up at local noon.

2.2. Biomass parameters

Replicate ($N = 3$), one per water table, colony nubbins were processed at three times during the five days of heat treatment (0900) for both *S. pistillata* and *P. cylindra* and once at the end of the 5 day recovery period.

The corals used in the experiment had no endolithic algae. Tissue was removed using a Waterpik and the homogenized slurry divided into aliquots for determination of symbiont density using replicate (8) hemocytometer counts and initial protein determination ($n=6$) for each species (Bradford, 1976). The skeleton was dried and the surface area was determined by the aluminum foil standard-curve technique (Marsh, 1970). Biomass determinations of experimental and control nubbins for both coral species were made on day 1. Following this the remaining replicate nubbins in the elevated temperature treatment were returned to ambient control temperatures of 28 ± 1 °C (mean ± SD) and sampled again on day 10.

2.3. Symbiont identification

A random selection of nubbins of *S. pistillata* ($N=6$) and *P. cylindrica* ($N=6$) were processed for their zooxanthellae as previously described (LaJeunesse, 2002). Nucleic acids were extracted from 20 to 40 mg of algal material using a modified Promega Wizard DNA prep protocol (Madison, WI) (LaJeunesse et al., 2003). PCR-denaturing gradient gel electrophoresis (PCR-DGGE) was used to analyze the internal transcribed spacer region 2 (ITS2) of nuclear ribosomal RNA genes. Comparison of this gene region effectively discriminates between different ecological types from within each major *Symbiodinium* clade (LaJeunesse, 2001, 2002; LaJeunesse et al., 2003). PCR was conducted using the "touchdown" amplification profile prescribed by LaJeunesse et al. (2003) and with the primers "ITSintfor2" (5′GAATTGCAGA ACTCCGTG-3′) and "ITS2CLAMP" (5′CGCCCGCCGC GCCCCGCGCC CGTCCCGCCG CCCCCGCCC GGGATCCATA TGCTTAAGTT CAGCGGGT-3′). Samples were loaded onto an 8% polyacrylamide denaturing gradient gel (45% to 80% urea-formamide gradient; 100% consists of 7 M urea and 40% deionized formamide) and separated by electrophoresis for 9.5 h at 160 V at a constant temperature of 60 °C (c.f. LaJeunesse, 2002). The gel was stained with SYBR Green (Molecular Probes, Eugene OR) for 25 min using the manufacturer's specifications, and photographed using 667 Polaroid film. The brightest bands from each distinctive fingerprint were then excised, re-amplified, and sequenced as previously described (LaJeunesse, 2002). ITS 2 sequences were aligned manually using Sequence Navigator version 1.0 software (ABI, Division of Perkin Elmer, Foster City, CA). The specific identity of each fingerprint profile was established by comparing the sequences with a database of symbionts previously characterized from this region (LaJeunesse et al., 2003) using maximum parsimony with PAUP 4.0b8 software (Swofford, 1993). Their identity was further verified by comparing each migration pattern with fingerprints from previously characterized *Symbiodinium* types (LaJeunesse et al., 2003).

2.4. Pulse-amplitude modulated fluorometry

Chlorophyll *a* fluorescence induction curves were obtained from the top of 15 vertically-oriented nubbins of both branching species of coral in each experimental tank ($n=3$) using data from a Pulse Amplitude Modulated fluorometer (Diving PAM, Waltz Germany) (total = 45 per species). Maximum light-dependent reductions in the steady state quantum yield of photosystem II ($\Delta F/Fm'$) were determined at local apparent noon (±20 min), while maximum quantum yields (Fv/Fm) were recorded at dusk (±20 min). The distance between the fiber optics of the fluorometer and the coral samples was maintained constant with a black plastic holder. Maximum excitation pressures over PSII were calculated as $Q_m = 1 - [(\Delta F/Fm' \text{ at noon}) / (Fv/Fm \text{ at dusk})]$ (Iglesias-Prieto et al., 2004).

2.5. Biochemical parameters

Tissue extracts for biochemical analysis were prepared by removing the coral tissues from the skeleton by airbrushing (80 psi, <1 cm distance from coral) in a low volume of filtered seawater (0.22 μm). The resulting mixtures were centrifuged at 16,110 ×*g* to remove any particulates and the soluble extract (supernatant) decanted into freshly labeled tubes and frozen at −80 °C.

2.6. Glycerol concentrations in coral tissue extracts

Coral tissue extracts for each treatment ($N=3$) sampling of both species were measured using the GPO Trinder diagnostic assay from Sigma Chemical Co (Procedure No. 337) as follows: 200 μl of each coral tissue extract was added to 600 μl of the reconstituted GPO Trinder Reagent and the mixture incubated for 10 min at room temperature prior to analysis. The absorbance of each of these mixtures at 540 nm was measured using a spectrophotometer zeroed using 200 μl of seawater mixed with 600 μl of the reconstituted GPO Trinder Reagent and the concentration of glycerol calculated using a standard curve generated with stabilized glycerol standards. The free glycerol concentrations were normalized to soluble protein, which was determined for the relevant tissue extract using the spectrophotometric assay of Whitaker and Granum (1980) standardized using gamma globulin.

2.7. Mycosporine-like amino acids

For all coral samples, the extraction and analysis of MAAs for each treatment ($N=3$) sampling of both species were performed according to the procedures described in Lesser (2000). For analysis by high performance liquid chromatography (HPLC), coral samples from the laboratory ($N=3$) were cleaned of epiphytes, broken into small pieces, and extracted overnight in 1 ml of 100% HPLC grade methanol at 4 °C. The extracts were centrifuged and the supernatant used for MAA and protein analysis. Individual MAAs were separated by reverse-phase, isocratic HPLC on a Brownlee RP-8 column (Spheri-5, 4.6 mm ID × 250 mm) which was protected with an RP-8 guard column (Spheri-5, 4.6 mm ID × 30 mm). The mobile phase consisted of 40 to 55% methanol (v:v), 0.1% glacial acetic acid (v:v) in water and run at a flow rate of 0.6 ml min^{-1}. Detection of MAA peaks was by measuring absorbance at 313 and 340 nm. Secondary standards were available for seven MAAs (mycosporine-glycine, shinorine, porphyra-334, palythine, asterina-330, palythinol, and palythene) and primary standards were available for shinorine, porphyra-334, and palythine. Identities of peaks were confirmed by co-chromatography with standards. Quantification of individual MAAs was accomplished by integrating individual HPLC peak areas after calibration using the standards listed above. All MAAs were normalized to soluble protein from an aliquot of the methanol-extracted sample and concentrations are expressed in nmol MAA mg $protein^{-1}$. Protein measurements were determined using the procedure of Bradford (1976) in kit form (Bio-Rad, Inc.).

2.8. Total lipids

Total lipids were extracted from ground coral samples ($N=3$) for each treatment ($N=3$) sampling of both species in a 2:1 chloroform: methanol solution, washed in 0.88% KCl followed by 1:1 methanol: water solution, then dried to constant weight (Harland et al., 1991; Grottoli et al., 2004). Total lipids were measured on whole ground coral samples (skeleton + coral host tissue + algal symbionts), normalized to the total ash-free dry weight of the organic fraction of the coral (host tissue + algal symbionts), and reported as g lipid gdw^{-1} as per Grottoli et al. (2004).

2.9. DNA damage

Cyclobutane pyrimidine dimer (CPD) formation was measured using the procedures and monoclonal antibody (TDM-2) originally

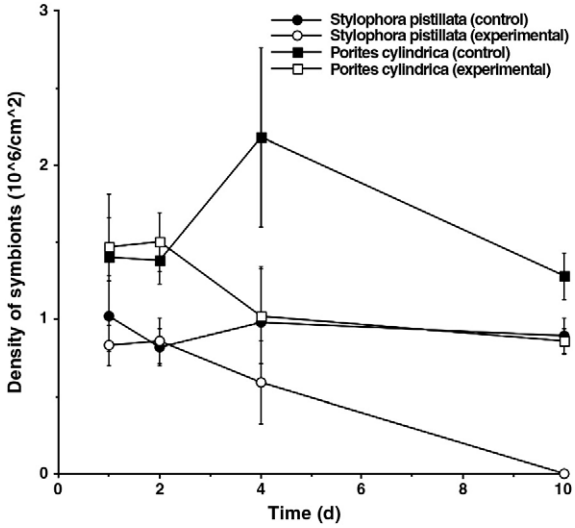

Fig. 1. Control and experimental zooxanthellae densities in *Stylophora pistillata* and *Porites cylindrica* during the 10 day experiment ($n=3$). The bar along the *X* axis represents the duration of exposure to 32 °C for the experimental treatment. All points are mean ± SE.

described by Mori et al. (1991) and as described for coral samples by Lesser and Farrell (2004). Coral DNA, both host and zooxanthellae, were isolated using commercially available kits (Quiagen, Inc.). Extracted DNA was quantified fluorometrically and 100 ng of DNA from each sample was used in an enzyme-linked immunoabsorbent assay (ELISA) technique with TDM-2 as the primary antibody. TDM-2 was freshly prepared from lyophilized aliquots of the monoclonal antibody that were used within 24 h of reconstitution. An affinity purified goat anti-mouse IgG secondary antibody conjugated with horseradish peroxidase was used and the final color development read in flat-bottomed 96 well plates using a plate reader (Bio-Rad, Inc.) at 490 nm as described by Mori et al. (1991).

2.10. Protein analysis

Samples of animal tissue and zooxanthellae ($n=3$) were collected at To and at T 5d by airbrushing using a small volume of phosphate buffer (2 mM, pH 7.5) and separated by centrifugation. The protocol for extraction, polyacrylamide gel electrophoresis (SDS-PAGE), and western blotting is described in Lesser and Farrell (2004). Briefly, SDS-PAGE gels of host tissues and zooxanthellae gels were run and electrophoretically transferred to PVDF membranes (0.2 μm). The membrane was then blocked with 3% instant milk and immunoblotting of the membrane was completed and developed using a secondary antibody at a titer of 1:2000 labeled with alkaline phosphatase or horseradish peroxidase. Differential expression of specific proteins for both the host tissues and zooxanthellae is accomplished by normalizing the loading of the gels on an equivalent total protein basis (Whitaker and Granum, 1980). The membranes were probed using polyclonal antibodies to detect Cu–Zn SOD protein (SOD I [cytosolic] 1:1000), and HSP 70 (Santa Cruz Biologicals, 1:100) in host samples while for protein extracted from zooxanthellae samples polyclonal antibodies against the D1 protein (1:1000) of PSII were used. In response to the experimental treatments, the differential expression of these proteins was analyzed by measuring the optical density of positive bands using NIH Image software. To remove differences in background staining and to quantify each band the pixel densitometry of positive bands and the area above and below each positive band was converted to optical density using an internal calibrated gray scale (0–255). This common procedure removes scan to scan instrument bias for a completely objective analyses. The optical densities are then compared statistically after log transformation.

2.11. Detection of coral mucus

Pieces of corals were analyzed histologically on day 0 (control), after 4 days at 32 °C, and again after 5 days of 32 °C, and after 5 days of recovery at 28 °C. They were fixed in paraformaldehyde and stained with toulidine blue and the mucopolysaccharide specific Periodic Acid Schiff's/Alcian Blue stain to visualize the mucus.

2.12. Statistical tests

The results of these experiments were analyzed using a two-way analysis of variance (ANOVA) with treatment and time as factors. If during an analysis time was found to not be significant the analysis was collapsed to a one way ANOVA to increase the power to detect treatment effects. All data were assed for normality and unequal variances. Where any were detected or in the case where results are *a priori* not normally distributed (e.g., ratios, optical densities) the values were log transformed for analysis and back transformed for presentation.

3. Results

Ambient downwelling (E_d) PAR irradiance was 2215 μmol quanta m^{-2} s^{-1} at local noon while measurements at the level of the coral nubbins were approximately 751 μmol quanta m^{-2} s^{-1}. Ambient UV-A irradiance was 53.2 W m^{-2} and UV-B was 2.29 W m^{-2} while at the coral nubbins it was 15.78 W m^{-2} and 0.57 W m^{-2} respectively at local noon. These means have ±3% variability over the field of downwelling exposure and are equivalent of the light field found 5–10 m deep.

3.1. Biomass parameters

Densities of symbiotic zooxanthellae at the beginning of the experiment (day 1) were about 1.0 (±0.2 se)×10^6 cm^{-2} for *S. pistillata* and 1.4 (±0.4 se)×10^6 cm^{-2} for *P. cylindrica* (Fig. 1). Densities of symbionts per unit surface area decreased approximately 20% during the first 4 days of high-temperature exposure for both species. Densities of symbionts in *S. pistillata* were near zero by day 10, and at least half of the experimental colonies had died, whereas none of the experimental or control colonies of *P. cylindrica* showed any visible evidence of partial or total mortality and had mean densities of symbionts between 0.9 and 1.2×10^6 cm^{-1} (ANOVA: $P<0.05$). The

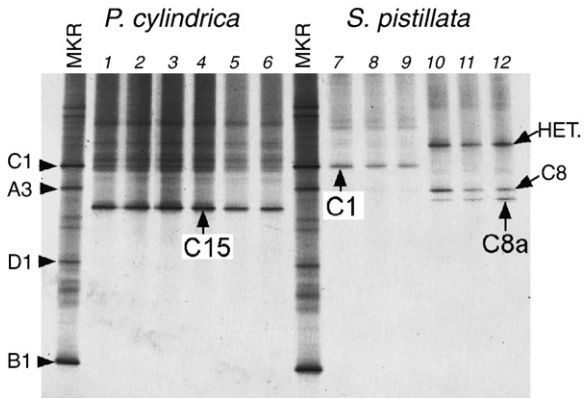

Fig. 2. PCR-DGGE profiles of zooxanthellae genotypes observed in samples of *Stylophora pistillata* and *Porites cylindrica* used during the experiment. Diagnostic bands appear as negative images and the alphanumeric name of each symbiont appears as uppercase letters indicate clade, number represents ITS type, and lowercase letters denote a characteristic rDNA paralog. MKR standards are pooled amplifications of C1, A3, D1, and B1. "Het." refers to the heterodublex formed by mismatching of complimentary DNA strands from C8 and C8a during the last PCR cycle.

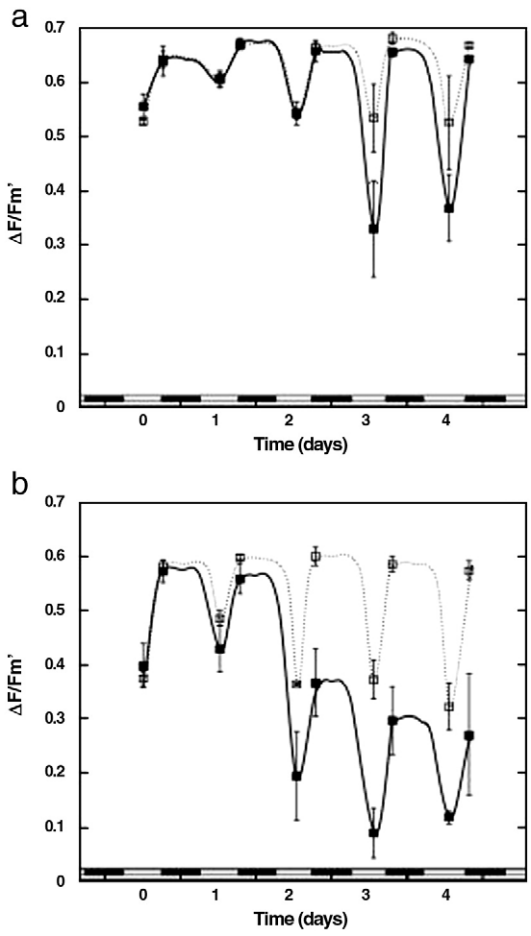

Fig. 3. Diurnal oscillations in the quantum yields of charge separation for photosystem II through the experimental exposure to elevated temperatures of the symbiotic dinoflagellates of (a) *Porites cylindrica* and (b) *Stylophora pistillata*. Dark squares represent either *Fv/Fm* (at dusk) or $\Delta F/Fm'$ (at noon) of control samples maintained at 26 °C. Open squares represent *Fv/Fm* (at dusk) or $\Delta F/Fm'$ (at noon) taken from corals exposed to 32 °C. Lines joining the fluorescence data are presented for clarity only. Error bars represent ± SE.

initial soluble protein was 16.35 ± 4.81 (sd) mg/cm^2 (n = 6) for *P. cylindrica* and 8.65 ± 2.96 (sd) mg/cm^2 (n = 6) *for S. pistillata.*

3.2. Symbiont identification

PCR-DGGE fingerprint profiles (Fig. 2) of *Symbiodinium* sp. types observed in *P. cylindrica* (lanes 1–6) and *S. pistillata* (lanes 7–12) showed that the dominant symbionts in *P. cylindrica* were type C15, while colonies of *S. pistillata* used in this experiment contained either type C1 or C8a (*sensu* LaJeunesse et al., 2003). It was not determined which symbiont (either C1 or C8a) each nubbin of *S. pistillata* had.

3.3. Active fluorescence

Control samples (pooled for dusk samples during the first 4 days) of *P. cylindrica* maintained at 26 °C showed significantly greater quantum yield of photosystem II (*Fv/Fm* values 0.667 ± 0.012, mean ± SE) than those values recorded for *S. pistillata* (0.591 ± 0.013) throughout the experiment (P<0.001 t-Student 2-tailed) (Fig. 3a, b). Although both species of coral were exposed to identical light fields, maximum excitation pressure over photosystem II (Q_m) experience by control samples were also significantly different for the duration of the experiment (0.170 ± 0.066, 0.348 ± 0.108 for *P. cylindrica* and *S. pistillata* respectively) (P<0.001 t-Student). Corals exposed to heat stress (32 °C) showed greater significant reductions in their steady state quantum yields ($\Delta F/Fm'$) at noon than control corals. Although these reductions were larger and appear earlier in the experiment in *S. pistillata* than in *P. cylindrica* (Fig 3a, b), thermal stress reduced the photochemical efficiency in both species. As a result of the observed reductions in energy conversion efficiencies, samples exposed to elevated temperatures showed dramatic increases in Q_m. Symbiotic dinoflagellates inhabiting both species experienced more than twice as much excitation pressure at noon relative to controls exposed to identical light fields after 4 days exposure to thermal stress. Despite these similarities, the patterns of recovery of *Fv/Fm* at dusk were different for each of the species. Thermal stress resulted in incomplete recovery of the maximum quantum yields of charge separation in the symbionts of *S. pistillata* after the first day of exposure to elevated temperatures. Reductions in *Fv/Fm* values (dusk samples of days 1–4) of the symbionts of *S. pistillata* showed a linear relationship with time of exposure to thermal stress ($Fv/Fm = 0.63 - (0.004)\text{Time(hrs)}$, $r^2 = 0.927$, P<0.05). These results indicate that under the experimental conditions employed here, the symbionts of *S. pistillata* lost 50% of their maximum quantum yields after 3 days. In contrast, samples (dusk samples of days 1–4) of *P. cylindrica* exposed to 32 °C showed a small but still significant linear reduction in the dark-adapted *Fv/Fm* as a function of time of exposure to elevated temperatures $Fv/Fm = 0.678 - (0.00033)\text{Time(hrs)}$, ($r^2 = 0.979$, P<0.05). As a result of the incomplete recovery of *Fv/Fm*, the symbionts of *P. cylindrica* would require more than 50 days exposure to the experimental temperature to experience a 50% reduction of initial quantum yield of charge separation. Associated with the reduction in

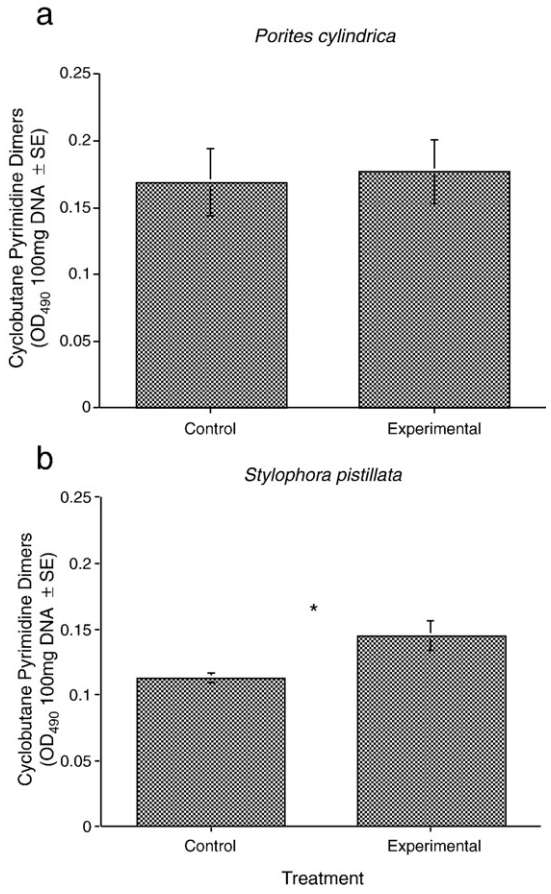

Fig. 4. a) Cyclobutane pyrimidine dimer concentrations (mean ± SE) in *Porites cylindrica*, and b) *Stylophora pistillata* after 5 days exposure to thermal stress compared to controls (n = 3).

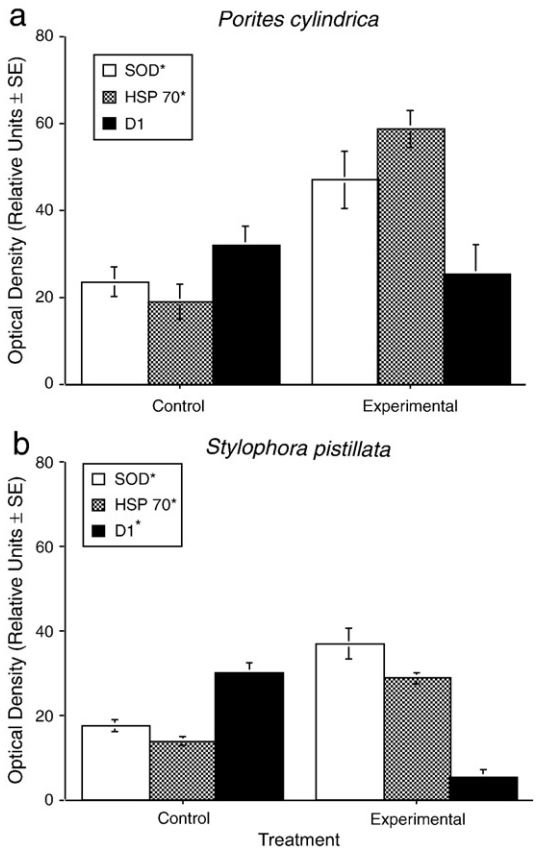

Fig. 5. a) Optical density (mean ± SE) of immunoblots in *Stylophora pistillata*, and b) *Porites cylindrica* after 5 days exposure to thermal stress compared to controls ($n = 3$).

Fv/*Fm*, there was an increase in mortality for samples of *S. pistillata* after exposure to 32 °C for 3 days.

3.4. Biochemical parameters

Glycerol concentrations in the tissue extracts of *P. cylindrica* ranged from 0.12–0.14 mg mg protein^{-1} and there were no effects of temperature (ANOVA: $P > 0.05$). Glycerol concentrations in *S. pistillata* exhibited a greater range of values (0.065–0.135 mg mg-protein^{-1}), and there were also no effects of temperature (ANOVA: $P > 0.05$). Additionally, there was no effect of species, day, or the interaction of species and day (ANOVA: $P > 0.05$).

P. cylindrica contained mycosporine-glycine, shinorine, porphyra-334, palythinol, asterina-330, and an unknown peak. These MAAs were quantified except for the unknown peak, which occupied 30–45% of the total peak area for both controls and experimental treatment groups. The presence of mycosporine-glycine, shinorine, porphyra-334, mycosporine-2 glycine, palythine-serine, mycosporine-NMA:serine, palythine, and palythinol was present in *S. pistillata* as described by Shick et al. (1999). All of these compounds were quantified except for mycosporine-2 glycine, which was consistently less than 5% of the total peak area in both control and experimental corals. The concentration of total MAAs for control and experimental treatment groups for both *P. cylindrica* and *S. pistillata* were not significantly different (ANOVA: $P > 0.05$). There were also no significant differences observed in total lipid content between control and experimental groups for either *P. cylindrica* or *S. pistillata*. DNA damage, measured as cyclobutane pyrimidine dimers (CPDs) were not significantly different between control and treatment samples of *P. cylindrica* (Fig. 4a), while for *S. pistillata* there was a significant difference between control and treatment (ANOVA: $P = 0.025$, Fig. 4b).

Immunoblots of host proteins in *P. cylindrica* and *S. pistillata* showed no significant difference between species at the beginning of the experiment (ANOVA: $P > 0.05$). At the end of the experiment, however, a significant treatment effect on the expression of both SOD (~16 kDa) and HSP 70 (~72 kDa) proteins after exposure to higher temperatures resulted in a significant increase in SOD (50% and 53% for *P. cylindrica* and *S. pistillata*) and HSP 70 (68% and 52% for *P. cylindrica* and *S. pistillata*) concentrations (ANOVA: $P < 0.05$, Fig. 5a, b). Immunoblots on the zooxanthellae of *P. cylindrica* for the 32 kDa D1 protein of PSII showed an insignificant 20% decline in concentration after exposure to elevated temperatures (ANOVA: $P > 0.05$, Fig. 5a), while in *S. pistillata* a significant 82% decline in D1 protein was observed (ANOVA: $P = 0.003$) when compared to controls not exposed to elevated temperatures (Fig. 5b).

Histology of *S. pistillata* tissue revealed the absence of mucocytes in epidermis after heat stress, while in deeper gastro-dermal tissue negligible quantity of mucus was present (Fig. 6b, d, f). In the case of *P. cylindrica* ample reserve mucus in form of mucocytes was obvious in epidermis (Fig. 6a, c, e).

4. Discussion

The results of this study clearly show both physiological and biochemical differences of both symbiont and host origin in the response to high-temperature stress of two species of branching corals. Photosynthetic processes, including fluorescence and concentrations of the thermally sensitive D-1 protein of photosystem II, were lower in the C1 and/or C8 *Symbiodinium* from *S. pistillata* than the values observed in the C15 *Symbiodinium* in *P. cylindrica*. In addition, the densities of symbionts in *P. cylindrica* were about 35% larger at the beginning of the experiment than those in *S. pistillata*, possibly due to greater host tissue biomass in the former. The decrease in *Symbiodinium* density in both species was about 20% over the first 4 days, but after the 5-day "recovery" period more than half of the experimental *S. pistillata* had died, while none of the controls or the *P. cylindrica* did. These results are similar to a number of different bleaching studies (e.g. Warner et al., 1999, Loya et al., 2001).

The impairment of the photosynthetic function of the symbionts was one of the first responses to thermal stress (Iglesias-Prieto et al., 1992; Lesser, 1996; Jones et al., 1998; Warner et al., 1999). This is manifested as a decrease in the dark-acclimated quantum yield of PSII fluorescence or *Fv*/*Fm* (Fig. 3). In the absence of other external stressors, *Fv*/*Fm* values are related to the light history of the sample (Rodríguez-Román and Iglesias-Prieto, 2005). For example, the quantum yields of PSII fluorescence in corals are inversely related to depth (Lesser and Gorbunov, 2001; Iglesias-Prieto et al., 2004). But the combination of high irradiances and thermal stress conditions, clearly showed that the symbionts of *P. cylindrica* were better able to dissipate the excess energy absorbed and repair the majority of damage to the photosynthetic apparatus compared to the symbionts in *S. pistillata* (Fig. 3).

Similar to other primary producers, symbiotic corals experience diurnal oscillations in their photochemical energy conversion efficiencies ($\Delta F/Fm'$) (Brown et al., 1999; Hoegh-Guldberg and Jones, 1999; Gorbunov et al., 2001). The light dependent reduction in $\Delta F/Fm'$ results in part from the induction of protective mechanisms that compete for the excited states of chlorophyll *a* and from the light-dependent inhibition of PSII. These two responses operate when the rate of light capture is larger than the rate of consumption of reducing power and ATP (Huner et al., 1999). Comparison of the reduction of $\Delta F/Fm'$ at noon in *S. pistillata* samples exposed to 32 °C relative to controls maintained at 28 °C, indicate that equal irradiance resulted in significant reductions of $\Delta F/Fm'$ under thermal stress. Similar results were obtained for *P. cylindrica*, although it required longer exposures to 32 °C. After 4 days, Q_m values were more than two times higher in samples exposed to elevated temperatures relative to controls in both

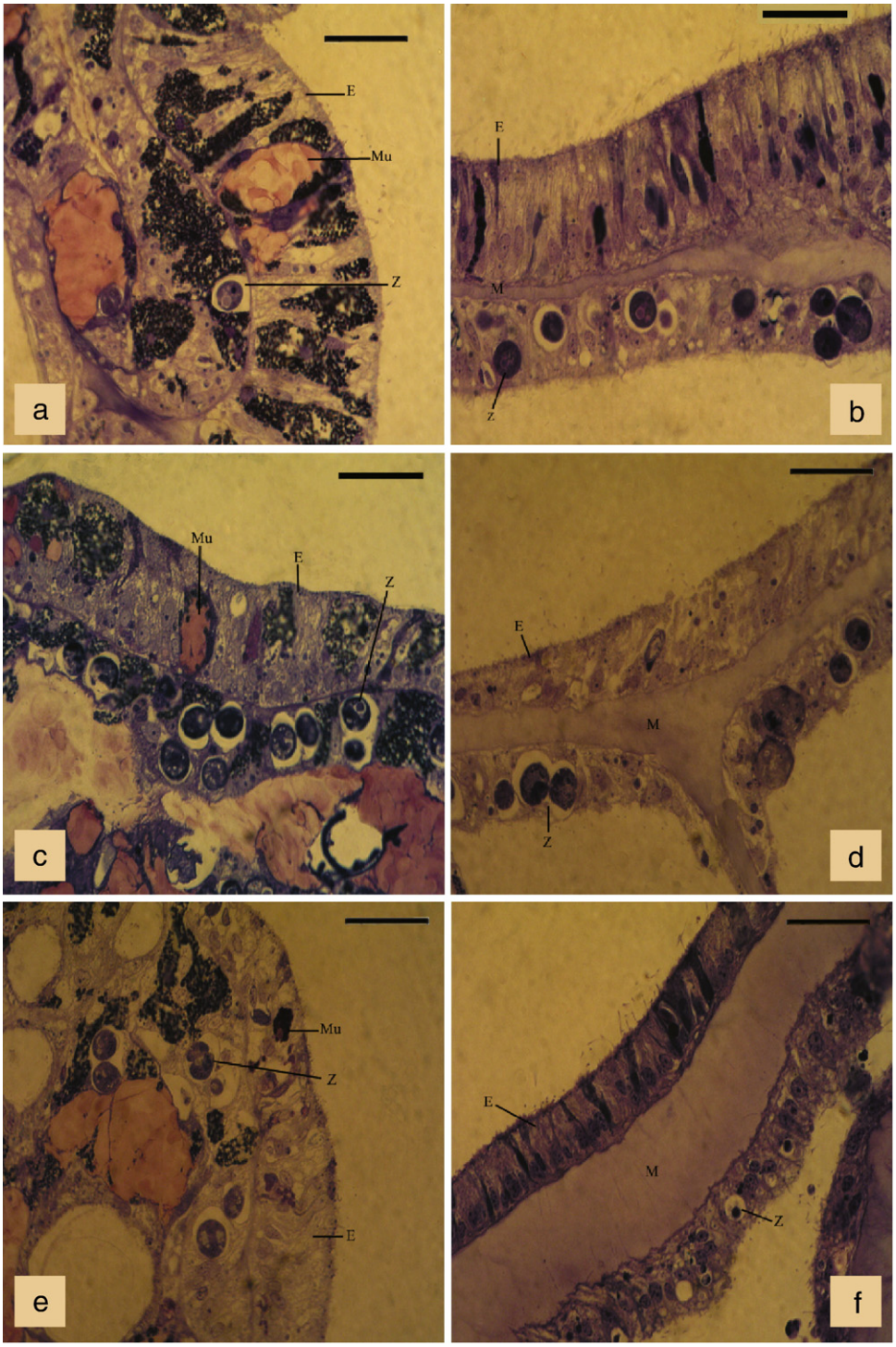

Fig. 6. Histology of heat-stressed *Porites cylindrica* tissue, showing ample reserve mucus in the form of mucocytes in epidermis at the beginning of the experiment (a) at the end of the heat-stress (c), and after an additional 5 days of recovery (e). In heat-stressed *Stylophora pistillata* the absence of mucocytes in epidermis is noticeable, while deeper gastro-dermal tissue has negligible quantity of mucus present at the end of the heat-stress (d), and after an additional 5 days of recovery (f) compared to the beginning of the experiment (b). Scale bar = 30 μM. Z = zooxanthellae, E = epidermis, Mu = mucocytes, M = mesoglea.

species. Q_m as employed here is a proxy measurement for non-photochemical quenching (NPQ) (Iglesias-Prieto et al., 2004). The results indicate exposure to elevated temperatures resulted in the impairment of the photosynthetic function in the dinoflagellates of both coral species. As a result of this inhibition, thermally stressed samples need to induce two times more NPQ relative to controls exposed to identical light fields. These reductions are consistent with a temperature-dependent impairment of PSII (Warner et al., 1999), and/or a reduced electron transport rate due to sink limitation (Jones et al., 1998). Under normal circumstances, *Fv*/*Fm* returns to its maximum value at night and the recovery of maximum quantum yields of PSII results from the relaxation of the photoprotective mechanisms and the efficient repair of damaged PSII (Melis, 1999). Warner et al. (1999) and Takahashi et al. (2004) suggested that the primary target of thermal stress in symbiotic dinoflagellates may be the PSII repair cycle itself. The results presented here are also consistent with temperature-dependent reductions in the rates of repair.

An additional source of variation in the response to thermal stress in these corals may originate from differences in tissue thickness (Warner et al., 2002) that was reflected in the difference in the initial protein content. The experimental corals were not significantly different (ANOVA: $P>0.05$) between species at the end of the heat stress for lipids, glycerol, and MAAs. Additionally, the levels of SOD and HSP 70 stress markers, and D1 protein were not significantly different between the two species of coral at the beginning of the experiment, but those of *P. cylindrica* were significantly higher in SOD and HSP 70 than *S. pistillata* at the end of the heat stress ($P<0.05$, ANOVA).

Differences in the response of these species of coral to thermal stress may also result from different *in hospite* irradiances driven by the combination of skeletal architecture and light scattering properties (Enríquez et al., 2005). Consistent with this interpretation, the maximum pressure over PSII (Q_m) in *S. pistillata* was two times larger than in *P. cylindrica*. An important point is that the different light environments within the tissues and skeletons of these corals may drive much larger physiological differences than simply the genetics of the symbiont. Given that photosynthetic physiology is at the heart of at least one of the problems faced by *Symbiodinium* as temperatures rise, the role of the host in determining the thermal tolerance of the association is far from trivial.

Ecological surveys suggest that the distribution of these two species of corals on the reef reflects their physiological capacities. *S. pistillata* was common along the outer slopes at all depths and was infrequently observed in the lagoon, while *P. cylindrica* was found in the lagoon and at leeward sites (van Woesik, unpublished data). These contemporary surveys also agree with an extensive analysis of coral communities in the southern Great Barrier Reef (van Woesik and Done, 1997). Clearly, the distribution of these two species of corals on the reef reflects their physiological capacities as outlined above, with *P. cylindrica* flourishing in the warmer back-reef and lagoon habitats while *S. pistillata* is found in a variety of habitats.

Whether the lack of mucus in *S. pistillata* relates to the species of *Symbiodinium* found in the host is not presently known. However, the mucus accounts for up to 45% of the total energy budget of the coral, and provides a first layer of defense for invading microbes (Brown and Bythell, 2005; Ritchie, 2006). The fact that *S. pistillata* has little or no mucus after 5 days of heat stress, followed by over half of the colonies dead after an additional 5 days in recovery, is quite different from mucus-filled *P. cylindrica* that are all alive at the end of the experiment.

Concentrations of HSP 70 and SOD protein increased during a 5 day exposure to thermal stress for both *S. pistillata* and *P. cylindrica*, but there was no significant change in the total concentration of MAAs, lipids, or glycerol. The significant increase in CPD formation for *S. pistillata* suggests that ultraviolet radiation may have added additional stress compared to *P. cylindrica* despite having similar concentrations of MAAs. This may indicate a decreased capacity for CPD repair (i.e., photolyase activity) under conditions of thermal stress. However, in *P. cylindrica* the response of expressed stress proteins is higher than those in *S. pistillata*, as is the repair of the critical PSII protein D1, and the combined effects of a thermally resistant symbiont type results in a more resistant holobiont phenotype. These results demonstrate that the characteristics of *Symbiodinium and* the response of the host are involved in short-term thermal tolerance. Hence, hypotheses that talk only in terms of the thermal characteristics of the symbiont may miss critical information concerning questions surrounding the thermal tolerance of corals in the coming century of rapid environmental change.

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