The physiological response of the reef coral *Pocillopora damicornis* to elevated temperature: results from coral reef mesocosm experiments in Southern Taiwan

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**A B S T R A C T**

Given the threat of climate change towards scleractinian corals, there is an urgent need to understand their physiological mechanisms of acclimation to increasing temperatures. To gain insight into this process, two mesocosm-based experiments were conducted in Southern Taiwan with the model reef-building coral *Pocillopora damicornis*. In the first study, temperature was gradually elevated to 32 °C, though reduced to ambient levels at night, in order to simulate a temperature profile that can characterize intertidal reefs of Southern Taiwan. All corals acclimated to such conditions over the course of the month-long experiment, as evidenced by a variety of physiological and sub-cellular responses. In the second experiment, corals were exposed continually to 31.5 °C for two weeks, and, in contrast to results from the first study, the majority of the corals died, revealing that prolonged exposure to this temperature is lethal for this dominant reef builder of many regions of the Pacific Ocean.

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1. Introduction

Coral reefs are currently threatened by rising levels of both temperature and pCO2 due to the well-documented environmental sensitivity of their framework-building organisms (Hoegh-Guldberg et al., 2007), reef-building corals engaged in an obligate endosymbiosis with dinoflagellates of the genus *Symbiodinium* (Stat et al., 2006). As such, there is an urgent need to better understand the physiological mechanisms of acclimation in these fragile endosymbioses. A wealth of information, in fact, exists on the scleractinian response to altered seawater quality, particularly increases in temperature (e.g., Hoegh-Guldberg and Smith, 1989). However, much of this prior work has focused on the response of corals in the field (e.g., Guest et al., 2012), in which it is typically not possible to establish a causal link between changes in certain seawater quality parameters and a particular physiological response due to uncontrollable heterogeneity in the natural environment. Furthermore, while microcosm-based studies (e.g., Coles and Jokiel, 1977) have greatly escalated what is known about the physiological response of corals (e.g., Franklin et al., 2004), they are inherently unrealistic, as they do not seek to mimic the marine ecosystems in which most corals live.

On the other hand, mesocosms potentially better approximate the natural habitat of the target species (Kuffner et al., 2007); in these studies, other taxa found to associate with the target species in the field are simultaneously cultured and exposed to the particular treatment of interest (e.g., Parsons, 1982). Marine biologists have utilized mesocosm systems for a number of years in order to understand, for instance, the ecosystem-level response to eutrophication (Taylor et al., 1995). Mesocosm-based approaches, albeit those employing very few functional groups, have also been used in the coral biology field (Andersson et al., 2009; Jokiel et al., 2008).
and will likely serve as a common tool for marine biologists in the coming years given the need to understand the response of coral reefs at both ecological and physiological scales to conditions they will face in the coming decades.

Given that the use of mesocosm-scale experimental manipulation systems could greatly enhance the current understanding of the coral response to elevated temperature, two experiments were conducted at Taiwan’s National Museum of Marine Biology and Aquarium (NMumba) mesocosm research facility (Liu et al., 2009) with the reef-building scleractinian Pocillopora damicornis. This species was chosen because it is amongst the most ubiquitously distributed in the world (Veron, 2000) and has been nominated to serve as a model coral for molecular work (Traylor-Knowles et al., 2011). Also, it the best-studied coral in Taiwan (e.g., Fan et al., 2002; Yeoh and Dai, 2010) and has, more recently, functioned as a model for global climate change (GCC) simulation studies (Putnam et al., in press).

The first study, referred to as the “nocturnal recovery experiment” (NRE) from hence forth, aimed to understand the coral response to fluctuating temperatures using a profile in which corals were exposed to elevated temperatures (up to 32 °C) during the day but ambient temperatures (27 °C) at night. It was hypothesized that, given half of each day to recover from elevated temperatures they routinely experience during low tides (Meng et al., 2008), the corals would acclimate to temperatures previously shown to result in bleaching and/or mortality in con-specifics from other regions (Barron et al., 2010). To compare the results of this study, a static temperature experiment, referred to as the “sustained increase experiment” (SIE) from hence forth, was then conducted, whereby corals were exposed to 31.5 °C for two weeks. As the temperature in this experiment was not reduced at night, it was hypothesized that the majority of the corals would bleach or die at these conditions aimed to simulate those predicated to characterize many reefs of the Indo-Pacific over the course of this century (Riahi et al., 2011).

Additional indices of coral performance: growth rate, Symbiodinium density, chlorophyll a (Chla) concentration, and the maximum quantum yield of photosystem II (Fv/Fm), as deduced from measurements obtained from a pulse amplitude modulating [PAM] fluorometer after dark adaptation), were also measured. Although it was hypothesized that corals of the NRE would perform similarly between temperature treatments, it was, in contrast, predicted that those exposed to 31.5 °C for two weeks (SIE) would demonstrate reductions in growth. Symbiodinium density, Chla concentration, and Fv/Fm based on the results of previous studies of other corals exposed to elevated temperatures for prolonged periods (e.g., Fitt et al., 2009; Jones et al., 2000).

In addition to these polyp/colony-level response variables, the molecular composition of the specimens was determined through extraction of their RNA, DNA, and protein. It was hypothesized that both RNA/DNA and protein/DNA ratios, which serve as proxies for total gene and protein expression, respectively, would change over time in samples of the SIE exposed to elevated temperatures given that expression of an additional suite of stress-response genes/proteins, such as heat shock proteins (hsp/HSPs), is necessary for the cellular stress response (Hochachka and Somero, 2002; Kultz, 2005). It was also hypothesized that absolute protein concentrations would decrease over time in samples exposed to 31.5 °C as a consequence of the hypothesized decrease in Symbiodinium density; briefly, protein content of corals has been shown previously to correlate with Symbiodinium density (Mayfield et al., 2011).

On the other hand, it was hypothesized that RNA/DNA and protein/DNA ratios, as well as areal protein concentration, would remain at similar levels in samples of both treatments of the NRE due to their predicted capacity to acclimate to the temperature regimes to which they were exposed.

From the RNA phase of samples of the SIE only, expression of one host and six Symbiodinium genes was assessed with real-time PCR. There was a particular focus on genes involved in photosynthesis given that photo inhibition has been repeatedly documented in corals exposed to elevated temperature (Smith et al., 2005). Specifically, expression of Symbiodinium ribulose-1,5-bisphosphate carboxylase/oxygenase (rbcL), phosphoglycerate phosphatase (pgpase), and photosystem I (psl, subunit III) was assessed with the expectation that their expression would decrease in samples exposed to 31.5 °C. Indeed, prior work has shown that exposure to elevated temperature can result in decreases in expression of the RBCl protein in endosymbiotic Symbiodinium (Putnam et al., in press) and symbiotic diatoms (Doo et al., 2012). Additionally, expression of a Symbiodinium nitrate transporter, nrt2, was also anticipated to decrease in response to exposure to 31.5 °C given the presumed impact of elevated temperature on the metabolic dialogue between the coral and its endosymbionts (Mayfield and Gates, 2007).

Expression of a Symbiodinium gene encoding a protein involved in detoxification of reactive oxygen species (ROS), ascorbate peroxidase (apx1, Shigeoka et al., 2002), was expected to demonstrate increased mRNA levels in corals exposed to the 31.5 °C treatment due to the fact that ROS have oftentimes been documented in thermally-challenged corals (e.g., Lesser, 1997). Similarly, expression of both the Symbiodinium and host coral hsp70 was measured and expected to be induced in samples exposed to elevated temperature given the fact that ROS resultant from photo inhibition could lead to protein denaturation and thus generate a demand for molecular chaperones, whose function involves the refolding of denatured proteins or prevention of their aggregation (Downs et al., 2002). Collectively, it was hoped that by measuring a variety of molecular parameters with methods that accommodate the dual-compartmental nature of coral tissues (sensu Mayfield et al., 2009), a more accurate and informative dataset would be unveiled that would allow for an enhanced degree of understanding of the sub-cellular mechanisms underlying the stress or acclimation responses in this important reef builder of the Pacific Ocean.

2. Materials and methods

2.1. Mesocosm design

Mesocosms (Fig. A1) were designed to be comprised of a similar assemblage of organisms at a similar density as found in Kenting National Park (KNP), the area from which the experimental organisms were collected (e.g., Yang, 1985). Analogously, the seawater quality conditions, most importantly temperature and light, were engineered to be similar to those measured in the field at the time of sampling (Meng et al., 2007a; Mayfield et al., 2012a). This work does not seek to document the field conditions from where the organisms described below were collected, though readers are encouraged to consult the extensive literature on both the ecology (e.g., Dai, 1991, 1993) and seawater quality (e.g., Meng et al., 2007b) of KNP, specifically Houwan Bay, as well as a prior work (Liu et al., 2009) discussing how the NMumba coral reef mesocosm facility attempts to simulate the marine environment of Southern Taiwan.

2.2. NRE

For a detailed explanation of how the mesocosms (Fig. A1) were constructed, see Appendix 1. Beginning in July 2011, all six mesocosms were programmed to be maintained at 27 °C, the approximate seawater temperature in the field at that time. On July 22, 2011, one coral nubbin was collected at approximately 16:00 from
each mesocosm \((t = 0 \text{ d})\). Then, three mesocosms were randomly chosen to serve as the control group, and the temperature profile was maintained at 27 °C for both the light (08:00–20:00) and dark (20:00–08:00) periods of each day. The experimental treatment mesocosms were brought to 30 °C over the course of one day, after which one nubbin was collected \((t = 1 \text{ d})\). Then, the temperature was reduced to 27 °C over the 12 h of darkness (Fig. 1A). This fluctuation, from 30 °C in the light to 27 °C in the dark, representing a temperature profile which can occur in shallow reef flats in Houwan Bay (Meng et al., 2007a), was continued for 7 d, after which an additional nubbin was collected at 16:00 \((t = 7 \text{ d})\). The next day, the temperature was elevated to 32 °C, and after 1 d of exposure to this temperature, an additional nubbin was collected from each mesocosm \((t = 8 \text{ d})\).

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**Fig. 1.** Temperature (A–B), photosynthetically active radiation (PAR; C–D), salinity (E–F), pH (G–H), and dissolved oxygen (DO; I–J) measured in the triplicate mesocosms of each of two treatments in the “nocturnal recovery” experiment (A, C, E, G, and I, respectively) and the “sustained increase” to 31.5 °C experiment (B, D, F, H, and J, respectively). In A, the gray solid and black dotted lines represent the high and control temperatures, respectively, for temperature logged at 10 min intervals. The symbols from all other panels represent data from seawater collected at the time of coral collection \((\sim 16:00)\). Error bars in B–J represent standard error of the mean (±SEM) for both control (hollow squares) and high (black diamonds) temperature samples. In B, F, I, and J, lower-case letters signify Tukey’s HSD groups \((p < 0.05)\) for the interaction effect of time and temperature, while in C, D, E, and H, they represent temporal differences only.
The temperature profile for the second week consisted of 32 °C during the light and 27 °C during the dark in order to simulate another temperature profile that can occur in the summer in intertidal regions of Southern Taiwan (Meng et al., 2007a). After 7 d of exposure to this profile, one nubbin was collected from each mesocosm at 16:00 (t = 15 d). Then, the temperature was reduced to 30 °C during the day and 27 °C at night for 3 d, after which an additional nubbin was sampled (t = 18 d). Finally, the temperature was set to 27 °C for the entire day, and after 1 d of exposure to 27 °C (t = 19 d), an additional nubbin was collected. One nubbin was collected on each of two additional days of this recovery period, 27 and 29 d after the start of the experiment.

2.3. SIE

The SIE, which was conducted in December 2011, consisted of the same mesocosms and assemblage/density of organisms (Table A1) as the NRE, though the same organisms were not used in both experiments. Specimens were collected from Houwan Bay in September 2011 and reared in the six mesocosms for three months at the temperature was set to 27 °C. Temperature was recorded at 10 min intervals with HOBO Pendant data loggers (Onset, MA, USA) in each of the six mesocosms in both experiments. Several additional seawater quality characteristics were measured at the same time (~16:00) at which P. damicornis nubbins were sacrificed. First, PAR (μmol photons m⁻² s⁻¹) was measured with a Li-193 sensor (Li-Cor, Lincoln, NE, USA) during the culture period, as well as after 0, 1, 15, 18, 19, 27, 29, and 31 d of experimentation for the NRE and after 0, 1, 7, 14, and 21 d of experimentation for the SIE. Salinity (psu) was measured with a CTD device (“conductivity, temperature, and depth,” Sea-Bird Electronics Model 19 plus, WA, USA) during the culture period, as well as after 0, 15, and 30 d of experimentation for the NRE, and after 0, 1, 7, 14, and 21 d for the SIE. Additional seawater was collected in 100 ml bottles at the time of coral collection for each experiment and measured in the laboratory as in Liu et al. (2012). Briefly, pH (total scale), dissolved oxygen (DO) concentration (mg l⁻¹), total alkalinity (TA, μmol kg⁻¹), and the concentrations of Chla (μg l⁻¹), nitrate (μmol kg⁻¹), nitrite (μmol kg⁻¹), and silicate (μmol kg⁻¹) were measured.

2.4. Seawater quality analysis

Temperature was recorded at 10 min intervals with HOBO Pendant data loggers (Onset, MA, USA) in each of the six mesocosms in both experiments. Several additional seawater quality characteristics were measured at the same time (~16:00) at which P. damicornis nubbins were sacrificed. First, PAR (μmol photons m⁻² s⁻¹) was measured with a Li-193 sensor (Li-Cor, Lincoln, NE, USA) during the culture period, as well as after 0, 1, 15, 18, 19, 27, 29, and 31 d of experimentation for the NRE and after 0, 1, 7, 14, and 21 d of experimentation for the SIE. Salinity (psu) was measured with a CTD device (“conductivity, temperature, and depth,” Sea-Bird Electronics Model 19 plus, WA, USA) during the culture period, as well as after 0, 15, and 30 d of experimentation for the NRE, and after 0, 1, 7, 14, and 21 d for the SIE. Additional seawater was collected in 100 ml bottles at the time of coral collection for each experiment and measured in the laboratory as in Liu et al. (2012). Briefly, pH (total scale), dissolved oxygen (DO) concentration (mg l⁻¹), total alkalinity (TA, μmol kg⁻¹), and the concentrations of Chla (μg l⁻¹), nitrate (μmol kg⁻¹), nitrite (μmol kg⁻¹), and silicate (μmol kg⁻¹) were measured.

Fig. 2. Bleaching (A–B), mortality, (C–D), and growth (E–F) assessed in Pocillopora damicornis samples reared within triplicate mesocosms of each of two treatments in the “nocturnal recovery” experiment (A, C, and E) and the “sustained increase” to 31.5 °C experiment (B, D, and F). Error bars represent ± SEM for both control (hollow squares) and high (black diamonds) temperature samples. In B and D, lower-case letters signify Tukey’s HSD groups (p < 0.05) for the interaction effect of time and temperature. In A and E, they represent temporal differences only.
2.5. Physiological parameter analysis

2.5.1. NRE

A PAM fluorimeter (Diving-PAM, Walz, Germany) was used to assess Fv/Fm in all nubbins both 7 and 1 d prior to experimentation, as well as after 2, 7, 10, 14, 19, 22, and 25 d of experimentation. Briefly, nubbins were dark-adapted for one hour prior to analysis, and the PAM settings of Mayfield et al. (2012a) were utilized for all measurements. Only Fv/Fm was assessed across all pseudo-replicated nubbins within each mesocosm at each sampling time, as this measurement does not require sacrificing the nubbins. During the experiment, two vision-based parameters, bleaching and mortality, were monitored. In short, the a priori definition of “bleaching” was when >50% of the tissue area of the nubbin had paled either from loss of *Symbiodinium* or their chlorophyll, and the percentage of the total number of remaining nubbins that had bleached (i.e., “% bleaching”) was calculated within each mesocosm. On the other hand, nubbins were considered dead if >90% of the tissue had either sloughed off or been overgrown by bacteria, macroalgae, or other non-coral flora, and a percentage of the total number of remaining nubbins that had died (i.e., “% mortality”) was calculated within each mesocosm at the time of sampling.

After 0, 1, 7, 8, 15, 18, 19, 27, and 29 d, 1 randomly selected nubbin out of the 12–14 in each mesocosm was removed at 16:00 and buoyantly weighed at the treatment temperature as described in Appendix 1. Then, tissue was removed from the skeleton with filtered seawater (FSW) emitted from a high-pressure water gun attached to a SCUBA cylinder, collected in plastic bags, and decanted into 50-ml centrifuge tubes. The tissue-less skeleton was then dipped in molten paraffin wax (65°C) in order to determine the surface area (SA) as in Stimson and Kinzie (1991). The change in mass (mg day⁻¹) was normalized to SA to yield mg cm⁻² d⁻¹.

One milliliter of tissue slurry was mixed with nine milliliters of acetone, incubated overnight at 20°C in the dark, and analyzed spectrophotometrically for *Chla* concentration, (μg ml⁻¹) as in Jeffrey and Humphrey (1975) after centrifuging the tubes at 12,000 × g for 15 min to pellet cellular debris. The total *Chla* quantity was then calculated by multiplying the concentration in 1 ml by the total volume of tissue slurry, which was variable across samples. Then, the total *Chla* quantity across the entire tissue slurry was normalized to SA to yield μg *Chla* cm⁻². From the same sample, 900 μl tissue slurry was removed and fixed with 100 μl 40% formaldehyde in FSW and stored at 4°C. *Symbiodinium* density was calculated from 10 replicate counts with a hemocytometer under light microscopy, multiplied by the tissue slurry volume, and normalized to SA to yield *Symbiodinium* cells cm⁻². The areal *Chla* concentration was divided by the cell density of the same sample to yield *Chla* concentration per cell (pg *Chla* cell⁻¹). Finally, 500 μl tissue slurry was mixed with 500 μl TRI-Reagent™ (Ambion, TX, USA) and frozen at −80°C for later extraction of RNA, DNA, and protein, as described in Appendix 2.

2.5.2. SIE

After 1 h of dark adaptation, Fv/Fm was measured in all nubbins with a PAM fluorometer as described above at the following times: 7 and 1 d prior to the start of the experiment and after 0, 7, 13, 15, and 20 d of experimentation. However, in this experiment Fv/Fm was also measured in the two additional corals, *Montipora stellata* and *Heliopora coerulea*, after 7, 13, 14, and 21 d of treatment exposure. Two *P. damicornis* nubbins were sacrificed after 0, 1, 7, 14, and 21 d. One nubbin was buoyantly weighed to estimate growth rate and processed as described above for analysis of *SA*, *Chla* concentration, and *Symbiodinium* density. From the second nubbin, a ~50 mg branch was removed and immersed in 500 μl TRIzol™ (Invitrogen, CA, USA) in a 1.5-ml microcentrifuge tube for later extraction of RNA, DNA, and protein, as described in Appendix 2.

2.6. Molecular analyses

For a detailed explanation of the molecular analyses, please see Appendix 2.

2.7. Statistical analyses

The biomass of organisms cultured within the six mesocosms for the SIE was compared between treatments with a student’s t-test when data were normally distributed and of equal variance and with a Wilcoxon rank-sum test when these conditions were not met. All other parameters, including seawater quality, physiology, molecular composition, and gene expression were assessed across treatments over time for both experiments, and, given that there was an expectation that the effects of the experimental treatment might become more pronounced with time, a 1-way, repeated-measures ANOVA was utilized with the JMP® statistical package. Statistically significant different differences are underlined.

### Table 1

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<th>Sustained increase</th>
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* a Analyzed by a univariate test due to lack of data sphericity.
* b Rank-transformed data.
* c Significant tank effect.
(Quinn and Keough, 2002). When the tank term was not statistically significant, it was dropped from the model. Tukey’s honestly significant difference (HSD) tests were used for post-hoc comparisons of individual means when significant differences were detected in the model.

3. Results

3.1. Seawater quality

For a detailed assessment of the seawater quality data from both studies, please see Appendix 3, Fig. 1 and Fig. A2, and Table A2.

3.2. Coral bleaching, mortality, and growth

3.2.1. NRE

The % bleaching (Fig. 2A) was low in samples of both treatments, ~7.5%, though the percentage increased significantly over time (Table 1). It did not, however, differ between treatments. Similarly, mortality (Fig. 2C) was low, ~5%, and did not differ significantly between treatments, over time, or in response to the interaction of temperature and time (Table 1). Finally, the growth rate (Fig. 2E) was similar in corals exposed to the two temperature treatments (Table 1), though it did change over time. Specifically, the growth rate approximately doubled from the 15th (~1 mg cm⁻² d⁻¹) to the 29th (~2 mg cm⁻² d⁻¹) sampling day.

3.2.2. SIE

Both % coral bleaching (Fig. 2B) and mortality (Fig. 2D) were significantly affected by temperature in the SIE (Table 1). After 14 d of exposure to 31.5 °C, 75% of the coral nubbins had bleached, versus only 7.5% of the control nubbins. After 7 d of recovery at 27 °C, the bleaching percentage decreased to similar levels in both treatments; however, this was because the previously bleached corals had died between these sampling times. As such, the % mortality of the high temperature treatment at 21 d (~80%) was significantly higher than that of the control treatment (~10%). In

Fig. 3. Symbiodinium density (A–B), areal chlorophyll a (Chla) concentration (C–D), Chla concentration per cell (E–F), and Fv/Fm (G–H), assessed in Pocillopora damicornis samples reared within triplicate mesocosms of each of two treatments in the “nocturnal recovery” experiment (A, C, E, and G) and the “sustained increase” to 31.5 °C experiment (B, D, F, and H). Error bars represent ± SEM for both control (hollow squares) and high (black diamonds) temperature samples. In H, lower-case letters signify Tukey’s HSD groups (p < 0.05) for the interaction effect of time and temperature, while in B–E and G, they represent temporal differences only. In A, B, G, and H, the first and second temperatures above brackets denote those of the control and experimental treatments, respectively, and the gray lines extend into lower panels in the former two panels to emphasize the temperature differences at each sampling time.
contrast, the growth rate (Fig. 2F) was not affected by treatment, time, or their interaction (Table 1) due to the fact that macroalgae were found to be overgrowing the dying symbionts of the high temperature treatment, hence increasing their mass.

3.3. Symbiodinium density, Chla concentration, and Fv/Fm

3.3.1. NRE

*Symbiodinium* density (Fig. 3A) was similar between treatments (Table 1), nor were there significant effects of time or the time × temperature interaction. On the other hand, areal Chla concentration (Fig. 3C) did vary significantly over time, though not in response to temperature or the temperature × time interaction (Table 1). Notably, the Chla concentration was 2-fold higher in nubbins sacrificed after 27 d of treatment exposure compared to the other sampling times. Chla per cell (Fig. 3E) also varied significantly over time (Table 1), and, specifically, decreased by ~50% after 15 and 18 d of exposure relative to other sampling times. Finally, Fv/Fm (Fig. 3G) also varied significantly over time, but not between treatments (Table 1).

3.3.2. SIE

The average *Symbiodinium* density (Fig. 3B) of samples from the SIE was similar to that of the NRE, ~0.5 × 10⁶ cells cm⁻². This parameter varied significantly over time (Table 1), though not between treatments. While areal Chla (Fig. 3D) varied significantly over time (Table 1), as well, cell-specific Chla content (Fig. 3F) did not. Regarding the former, areal Chla was found to be ~2-fold higher in nubbins collected after 7 d of exposure relative to those sampled at 21 d.

Fv/Fm (Fig. 3H) demonstrated a statistically significant treatment × time effect (Table 1). Specifically, it began to decrease in elevated temperature samples after 7 d of exposure to 31.5 °C and continued to decrease even after 6 d of recovery (t = 20 d). Fv/Fm was also measured in *M. stellata* (Fig. A3A) and *H. coerulea* (Fig. A3B) and responded significantly to temperature in the latter (Table 1). Specifically, Fv/Fm decreased in blue coral samples assessed after 14 d of exposure to elevated temperature, and there was an approximate 50% decrease in experimental samples relative to controls at this sampling time. Fv/Fm increased during the recovery period and reached similar values as the controls after 7 d of recovery (t = 21 d). Finally, although both time and the time × temperature interaction significantly affected Fv/Fm values of the tabular, reef-building coral *M. stellata* (Table 1), there were no post-hoc differences, and, in general, the differences were less pronounced than those observed in *P. damicornis* and *H. coerulea*.

3.4. Coral molecular composition

3.4.1. NRE

Areal DNA (Fig. A4A) and RNA (Fig. A4C) concentrations were both stable across time and treatments (Table 2). On the other hand, areal protein concentration (Fig. 4B) changed significantly over time (Table 2) and was notably higher in samples sacrificed after 27 d, representing the samples that also possessed the highest *Symbiodinium* densities. Neither the RNA/DNA (Fig. 4C) nor the protein/DNA (Fig. 4E) ratio varied in response to temperature treatment, time, or their interaction (Table 2).

3.4.2. SIE

Areal protein concentration (Fig. 4B) was similar between treatments and stable over time in samples of the SIE (Table 2). Furthermore, neither RNA/DNA (Fig. 4D) nor protein/DNA (Fig. 4F) ratios were affected by temperature, time, or their interaction (Table 2). Finally, although there appeared to be a significantly higher *Symbiodinium* genome copy proportion (GCP; Fig. A4B), and consequently significantly lower host GCP (Fig. A4D), in samples sacrificed after 14 d of treatment exposure, these differences were not statistically significant, and, in sum, there were no effects of temperature, time, or their interaction on these parameters (Table 2).

3.5. Symbiodinium diversity

Table 2

<table>
<thead>
<tr>
<th>Parameter (source of variation)</th>
<th>Nocturnal recovery</th>
<th>Sustained increase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>p</td>
</tr>
<tr>
<td><strong>Areal RNA (µg cm⁻²)</strong></td>
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<tr>
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<td>0.712</td>
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<td>Time</td>
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<tr>
<td><strong>Areal DNA (µg cm⁻²)</strong></td>
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<td></td>
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<tr>
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<td>0.935</td>
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<tr>
<td>Time</td>
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<td>0.595</td>
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<tr>
<td>Temperature × Time</td>
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<tr>
<td><strong>Areal protein (mg cm⁻²)</strong></td>
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<tr>
<td>Time</td>
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<td><strong>Symbiodinium genome copy proportion</strong></td>
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<tr>
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<td><strong>Protein/DNA ratio</strong></td>
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<td>Temperature</td>
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<td>0.603</td>
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<tr>
<td>Time</td>
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<td><strong>P. damicornis genome copy proportion</strong></td>
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<tr>
<td>Temperature</td>
<td>0.997</td>
<td>0.446</td>
</tr>
</tbody>
</table>

a Rank-transformed data.
b Log-transformed data.
c Square-root-transformed data.
d Analyzed by a univariate test due to lack of data sphericity.

3.5.1. NRE

Table 2 One-way, repeated-measures ANOVA of molecular composition parameters assessed in the “nocturnal recovery” (NRE) and “sustained increase” (SIE) experiments. Statistically significant differences are underlined.

The quantity of the exogenous RNA spike that was reverse transcribed was quantified with real-time PCR (Fig. 5A) and found to be stable across treatment and time (Table 3). Expression of three photosynthesis-targeted genes (PTGs); *rbcL*, *psII*, and *papase* (Fig. 5C), and *psl* (Fig. 5D), one encoding a protein involved in ROS detoxification, *apx I* (Fig. 5E), and one encoding a protein involved in nitrate transport (*nrt2*, Fig. 5F) was measured and found to be stably expressed across treatments and time (Table 3). Likewise, expression of the molecular chaperone *hsp70* was measured in both *Symbiodinium* (Fig. 5G) and the host coral *P. damicornis* (Fig. 5H).

3.6. Gene expression

In no sample from either treatment at any sampling time was a positive amplification (C new < 30) detected for clade A or D *Symbiodinium*. On the other hand, all 30 DNAs of the SIE were readily amplifiable with the clade C primer set, suggesting that only *Symbiodinium* within this clade were associated with these *P. damicornis* samples.
and, in both compartments, was found to be expressed at stable levels across treatments and time (Table 3).

4. Discussion

In contrast to previous studies (e.g., Vidal-Dupiol et al., 2009), it was found herein that P. damicornis can acclimate to elevated temperatures up to 32 °C provided that the temperature is reduced to ambient levels for at least half of each day. Specifically, growth, Symbiodinium density, Chla content, RNA/DNA and protein/DNA ratios, and protein concentration did not vary between treatments at any sampling time in the NRE, though the majority of these parameters did change over time (discussed in greater detail below). On the other hand, specimens of the high temperature treatments of the SIE demonstrated significant increases of two treatments in the “nocturnal recovery” experiment (A, C, and E) and the “sustained increase” to 31.5 °C experiment (B, D, and F). Error bars represent ± SEM for both control (hollow squares) and high (black diamonds) temperature samples. In A and B, the first and second temperatures above brackets denote those of the control and experimental treatments, respectively, at each sampling time. Gray lines extend into lower panels to emphasize the temperature differences at each sampling time in each experiment.

Curiously, other parameters used to infer a degree of coral health, such as Symbiodinium density and Chla concentration, did not reveal similar reductions in samples of the SIE. In fact, after one and two weeks of elevated temperature exposure, Symbiodinium still residing within gastrodermal tissues possessed significantly higher Chla levels on a per-cell basis, perhaps as an adaptive response to the absence of shading from other Symbiodinium that had been lost (Gorbunov et al., 2001). Given that Chla per cell did not decrease, though corals paled visibly, it stands that P. damicornis populations in Taiwan may bleach via loss of dinoflagellates (as opposed to only loss of Chla), as has been documented in conspecifics from Panama (D’Croz and Mate, 2004). That being said, Symbiodinium density did not differ significantly between treatments of the SIE. It is possible that, as fixation for several days in formaldehyde can cause pigment loss, non-Symbiodinium algae, which were growing on most of the bleaching nubbins and not removed from the nubbins prior to tissue removal, were inadvertently quantified due to the majority of the cells being similar in size and of a similar color. As such, the Symbiodinium density and Chla data from these bleached samples must be interpreted with caution. Additionally, this finding points to one caveat with using mesocosms: physiological analyses may be biased due to interactions between the cultured organisms within each mesocosm. Such a disagreement between visible degrees of coral health decline and stress-indicative patterns of physiological response...
variables also extends to the molecular data, which were, in general, markedly similar between treatments of the SIE. Specifically, RNA/DNA and protein/DNA ratios, as well as areal protein concentration, were similar between treatments, suggesting that their overall molecular composition did not differ. Furthermore, *Symbiodinium* and host GCPs, as well as the *Symbiodinium* identity, were also similar between treatments. Finally, expression of none of the six target *Symbiodinium* genes was affected by temperature, in contrast to what was hypothesized. Even PTGs such as *psI* and *rbcL* were expressed at similar levels between treatments, despite the fact that *Fv/Fm* had declined in these samples.

Given the fact that all genes targeted herein demonstrated similar expression levels between treatments, we hereby reject their potential to serve as biomarkers for coral health assessment. Other studies have found that extensive temporal variation in coral gene expression (Levy et al., 2011; Mayfield et al., 2010), as well as their physiology in general, caused by metabolic changes that vary as a function of the light cycle (Levy et al., 2006; Mayfield and Gates, 2007) may also confound the ability to use expression levels of certain macromolecules to infer a degree of coral health; such temporal variation in coral physiology was indeed documented herein and in numerous prior works (e.g., Levy et al., 2006).

Specifically, upon a cumulative assessment of all data of the NRE pertaining to physiology (Table 2) and molecular composition (Table 3) it is evident that, in most cases, temporal variation was far more significant than treatment-derived variation. For instance, all physiological parameters documented in Table 2 (with the exception of mortality and *Symbiodinium* density) differed significantly over time, including the rate of growth. Similarly, both *Chla* concentration (per cell only) and *Fv/Fm* (in *P. damicornis* and *M. stellata*) differed over time in the SIE, possibly due to the deliberate reduction in light levels exerted in order to simulate the shortening of days that was occurring over the course of the winter study. In the field, it has indeed been shown that coral *Chla* content and *Symbiodinium* density vary seasonally (Stimson, 1997), and thus presumably as a function of temperature, integrated PAR, and other seawater quality parameters that vary extensively over a long-term timescale. Furthermore, the tissue composition of coral has also been shown to vary over both long-term (Fitt et al., 2000) and short-term (Mayfield et al., 2012b) timescales, though only areal protein concentration was shown herein to be temporally variable within an experiment.

Given such temporal changes both within and between experiments, it is recommended that future studies seeking to simulate
the field environment over a multi-week timescale or those looking to compare results from experiments conducted during different seasons carefully judge how environment differences may affect the physiological response of the target organism and whether such differences may differentially affect the respective treatment groups. It should also be noted in such studies that changes in environmental parameters such as pH and DO (Fig. 1), and such collective shifts in seawater composition induced by temperature, can significantly influence the physiological response of the target organism and whether it is capable of adapting to thermal stress. Hottinger et al. (2012) demonstrated that bleaching susceptibility in 2010 suggests an adaptive response to thermal stress. PLoS ONE, e50685.ollider’s thermal stress in corals without changes in symbiont composition. Proceedings of the Royal Society B: Biological Sciences 279, 1020–1027.


Further reading

