The Journal of Experimental Biology 215, 4183-4195

doi:10.1242/jeb.071688

RESEARCH ARTICLE

The effects of a variable temperature regime on the physiology of the reef-building coral *Seriatopora hystrix*: results from a laboratory-based reciprocal transplant

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SUMMARY

To understand the effects of global climate change on reef-building corals, a thorough investigation of their physiological mechanisms of acclimatization is warranted. However, static temperature manipulations may underestimate the thermal complexity of the reefs in which many corals live. For instance, corals of Houbihu, Taiwan, experience changes in temperature of up to 10°C over the course of a day during spring-tide upwelling events. To better understand the phenotypic plasticity of these corals, a laboratory-based experiment was conducted whereby specimens of *Seriatopora hystrix* from an upwelling reef (Houbihu) and conspecifics from a non-upwelling reef (Houwan) were exposed to both a stable seawater temperature (26°C) regime and a regime characterized by a 6°C fluctuation (23–29°C) over a 12 h period for 7 days. A suite of physiological and molecular parameters was measured in samples of both treatments, as well as in experimental controls, to determine site of origin (SO) and temperature treatment (TT) responses. Only chlorophyll *a* (chl *a*) concentration and growth demonstrated the hypothesized trend of higher levels when exposed to a TT that mimicked SO conditions. In contrast, chl *a*, maximum dark-adapted quantum yield of photosystem II (F_v/F_m), and *Symbiodinium* ribulose-1,5-bisphosphate carboxylase/oxygenase (*rbcL*), photosystem I (*psI*, subunit III) and phosphoglycolate phosphatase (*pgpase*) mRNA expression demonstrated significant TT effects. Specifically, levels of these response variables were higher in samples exposed to a variable temperature regime, suggesting that *S. hystrix* may acclimate to fluctuating temperatures by increasing its capacity for photosynthesis.

Supplementary material available online at http://jeb.biologists.org/cgi/content/full/215/23/4183/DC1

Key words: acclimation, coral reef, endosymbiosis, gene expression, photosynthesis, *Symbiodinium*.

Received 12 April 2012; Accepted 20 August 2012

INTRODUCTION

The effects of global climate change on coral reef ecosystems are becoming increasingly apparent (Hoegh-Guldberg, 1999) and have not only ecological (Hoegh-Guldberg et al., 2007), but also socio-economic implications (Hughes et al., 2003). As such, there is an urgent need to better understand the thermal biology of reef-building corals (Jokiel and Coles, 1990; Gates and Edmunds, 1999; van Oppen and Gates, 2006; Edmunds and Gates, 2008), as well as more fundamental components of their cellular (Chen et al., 2012) and sub-cellular behavior (Mayfield and Gates, 2007; Peng et al., 2011). Unfortunately, traditional temperature manipulation studies of corals are oftentimes characterized by shortcomings that prevent us from making strong claims about coral responses to elevated temperatures under environmentally relevant conditions. Importantly, experiments have typically challenged corals with a single elevated temperature for brief periods in an attempt to simulate an acute thermal stress event (e.g. DeSalvo et al., 2008; but see Dove, 2004; Putnam and Edmunds, 2009; Putnam et al., 2010; Oliver and Palumbi, 2011; Putnam and Edmunds, 2011). However, many coral reefs experience large variations in temperature (Craig et al., 2001; Leichter et al., 2005; Leichter et al., 2006; Sheppard, 2009), and some even undergo dramatic temperature changes over a time scale of minutes to hours. For instance, coral reefs of Nanwan Bay, the southernmost embayment of Taiwan, experience episodic upwelling (Lee et al., 1999) whereby the temperature may change up to 10°C over the course of a summer day during spring tides (Jan and Chen, 2008).

As thermal heterogeneity can be high within the seawater surrounding certain reefs, the use of static temperature manipulations alone may minimize the ability to interpret the physiological response of corals inhabiting such thermally variable regions. Reciprocal transplants *in situ* (e.g. Fan and Dai, 1999; Smith et al., 2007; Smith et al., 2008; Barshis et al., 2010; Bongaerts et al., 2011), in contrast, stand to greatly enhance our understanding of the phenotypic plasticity of corals (Coles and Brown, 2003), as they inherently avoid potentially unrealistic thermal regimes. In fact, thermal history has repeatedly been shown to influence the physiological response and acclimation capacity of reef corals (Coles, 1975; Coles and Jokiel, 1978; Warner et al., 1996; D'Croz and Mate, 2004; Castillo and Helmuth, 2005; Middlebrook et al., 2008; Howells et al., 2011; Oliver and Palumbi, 2011; Carilli et al., 2012; Guest et al., 2012), further promoting the utility of reciprocal transplants in developing our understanding of the coral response to changing temperatures. However, field-based studies are

susceptible to impact by unexpected environmental variability, and results must always be interpreted conservatively.

With this in mind, by conducting a laboratory-based reciprocal transplant (LBRT) study, we took advantage of the thermally unique and dynamic environments of southern Taiwan in order to gain insight into how a common reef-building scleractinian, *Seriatopora hystrix* Dana 1846, acclimates to changes in seawater temperature. Coral colonies were collected from both Houbihu, a reef within Nanwan Bay that experiences episodic summer upwelling (Putnam et al., 2010), and Houwan, a reef on the western side of the Hengchun Peninsula that does not experience this phenomenon, and specimens from each site were incubated at either stable (26°C) or variable (23–29°C over a 12 h period) temperature for 7 days. This laboratory-based approach was also utilized in place of a reciprocal transplant *in situ* because of the multitude of other abiotic parameters (e.g. nutrient and dissolved oxygen levels) that are affected by upwelling events in Taiwan (Chen et al., 2004); while the physiological response to upwelling is indeed a worthy avenue for future research, only the temperature changes associated with such oceanographic events were of interest in this work. Collectively, it was hypothesized that corals exposed to upwelling conditions in nature would perform better under a variable temperature regime in the laboratory in comparison to those that experience relatively more stable annual temperatures. In other words, in the absence of acclimation, transplanted corals were expected to be physiologically compromised relative to non-transplanted controls, as corals have repeatedly been shown to be particularly sensitive to changes in temperature (e.g. Hoegh-Guldberg and Smith, 1989; Gates, 1990; Fitt and Warner, 1995; Hoegh-Guldberg and Jones, 1999).

The effects of temperature changes on reef coral physiology, and particularly photosynthesis, are relatively well documented (Jones et al., 1998; Warner et al., 1999; Fitt et al., 2001; Smith et al., 2005; Venn et al., 2008; Weis, 2008). Exposure to dramatically elevated temperatures results in photoinhibition of the endosymbiotic dinoflagellates (genus *Symbiodinium*) within corals (Jones et al., 2000). In extreme circumstances, *Symbiodinium* may be lost from the coral tissues and/or display reductions in chlorophyll *a* (chl *a*) concentration, phenomena known as bleaching (Glynn, 1983). While significant levels of bleaching were not anticipated for transplanted corals of this study due to the use of temperatures below the locally reported bleaching threshold of *S. hystrix* (Hung et al., 1998; Mayfield et al., 2011), decreased *Symbiodinium* densities and chl *a* concentration were hypothesized to be displayed by transplanted corals. In addition, it was anticipated that corals exposed to altered thermal regimes would demonstrate a decrease in maximum dark-adapted quantum yield of photosystem II (PSII) (F_v/F_m), as seen in previous studies (Putnam and Edmunds, 2009; Putnam and Edmunds, 2011). Given both the coral dependence on *Symbiodinium* photosynthesis for organic carbon (Muscatine and Cernichiari, 1969; Muscatine et al., 1981) and the strong connection between *Symbiodinium* photosynthesis and coral calcification (Gattuso et al., 1999), a reduction in both *Symbiodinium* density and F_v/F_m in transplanted corals would be expected to lead to decreased growth in these same samples.

Photoinhibition initially manifests at the molecular level (Shapira et al., 1997), and it was hypothesized that *Symbiodinium* within transplanted corals would express decreased levels of two photosynthesis-targeted genes (PTGs) encoding proteins involved in photon capture and trafficking [photosystem I (*psI*, subunit III)] and the Calvin cycle [ribulose-1,5-bisphosphate carboxylase/oxygenase (*rbcL*)]. Although expression of *Symbiodinium psI* and *rbcL* have not been measured in reef corals, expression of the third PTG gene targeted herein, phosphoglycolate phosphatase (*pgpase*), which encodes a photorespiratory protein involved in removal of the photosynthetic by-product 2-phosphoglycolate (Husic and Tolbert, 1985; Mamedov et al., 2001), was previously documented to decrease in *Symbiodinium* within corals that had been exposed to elevated CO_2 partial pressure (Crawley et al., 2010), and the authors interpreted this to signify that those corals were photosynthetically compromised and hence unable to fix organic carbon at optimal rates. However, as photorespiration is also a mechanism of photochemical quenching (Heber et al., 1996), it is possible that corals may utilize this pathway as a mechanism to increase energy dissipation when experiencing stress-induced photoinhibition in order to avoid reactive oxygen species (ROS) production (Gorbunov et al., 2001), discussed in greater detail below. Therefore, unlike *rbcL* and *psI*, *pgpase* gene expression was hypothesized to increase in *Symbiodinium* in corals exposed to a temperature treatment (TT) different from that of their site of origin (SO).

Photoinhibition-derived ROS production has repeatedly been observed in corals exposed to elevated temperatures (e.g. Lesser, 1997; Downs et al., 2002), and it has been identified as a major factor eliciting the coral bleaching response (Franklin et al., 2004). As such, ascorbate peroxidase (APX1), which is a key enzyme required for the detoxification of ROS (Shigeoka et al., 2002) in algae and higher plants (Yoshimura et al., 2000), would potentially be induced in photosynthetically compromised *Symbiodinium*. Given the expectation for photoinhibition in transplanted corals, it was also hypothesized, then, that expression of the mRNA encoding APX1, *apx1*, would increase in transplanted corals. Finally, transplanted corals were also hypothesized to demonstrate elevated RNA/DNA and protein/DNA ratios, indicative of increased levels of gene and protein expression and turnover, respectively, which may be necessitated by the ROS (Lesser, 2006) and, more generally, cellular defense responses (Hochachka and Somero, 2002; Kültz, 2005). Collectively, it was hoped that by measuring both physiological and molecular parameters with methodologies that account for the endosymbiotic nature of the coral tissues (*sensu* Mayfield et al., 2011), a greater understanding of the coral response to altered temperature regimes would be obtained.

MATERIALS AND METHODS

Field data acquisition and coral sampling

Five months prior to sampling (January 2010), HOBO Pendant data loggers (Onset, Pocasset, MA, USA) programmed to record temperature at hourly intervals were left at both Houwan (22°01′23.30″N, 120°41′18.29″E, non-upwelling site) and Houbihu (21°56′18.01″N, 120°44′45.54″E, upwelling site). The average temperature logged at Houwan between April and June 2010 (~26°C, Fig. 1A,B) was used as the ‘stable’ temperature treatment (Fig. 1C). Temperatures logged during spring-tide upwelling events occurring at Houbihu between May and June 2010 (Fig. 1D,E) were used to set the ‘variable’ temperature treatment used in the study (Fig. 1F), described below. Photosynthetically active radiation (PAR) was measured hourly on SCUBA with a cosine-corrected Li-Cor meter (LI-193 attached to a LI-1400 data logger *via* a 10 m cable; Li-Cor Biosciences, Lincoln, NE, USA) from 06:00 to 20:00 h (N=15 sampling times per day) at the approximate site of coral collection (7–8 m depth) on two non-overcast summer days prior to sampling and one non-overcast day following the sampling day at each of the two sites in order to estimate the average PAR level (μmol photons m^{-2} s^{-1}) experienced by the *S. hystrix* colonies

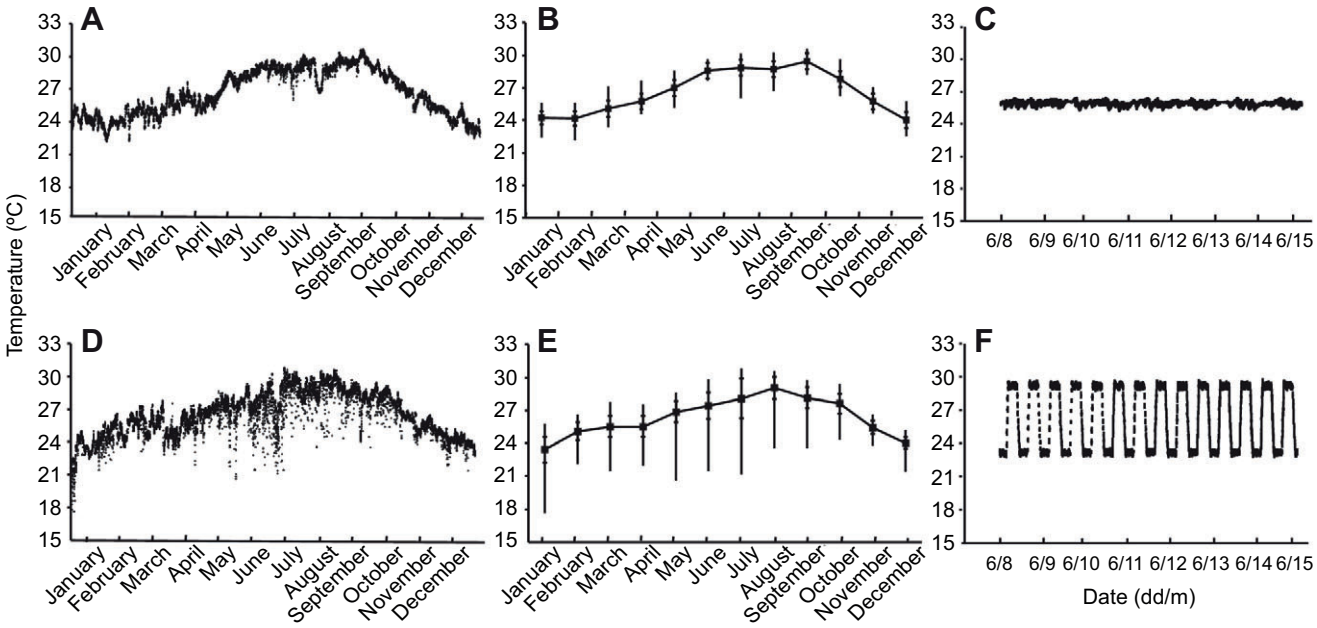

Fig. 1. Field and experimental temperatures. Temperature was measured hourly with HOBO Pendant data loggers for 1 year (2010) at the two sites from which the corals were collected: (A,B) Houwan (non-upwelling site) and (D,E) Houbihu (upwelling site). (B,E) Monthly mean temperatures; internal hatches and longer error bars represent the standard deviation (s.d.) and range, respectively. The 12 coral colonies used in this study were collected in May. (C,F) Temperatures in each of the six stable (C; mean ± s.d.) and six variable (F; mean ± s.d.) tanks, recorded at 10 min intervals with HOBO Pendant data loggers. In most instances, the error bars do not extend beyond the symbol.

in situ. The mean (±s.d.) hourly values obtained across both sites (94.3±8.6 and 94.3±9.1 μmol photons m^{-2} s^{-1} for the non-upwelling and upwelling sites, respectively) were used to set the PAR level used in both the acclimation and experimental periods (~90–100 μmol photons m^{-2} s^{-1}).

In May 2010, under Kenting National Park permit 0992900398, six *S. hystrix* colonies were removed on SCUBA from each site (7–8 m depth) and transported to Taiwan's National Museum of Marine Biology and Aquarium. The colonies were allowed to acclimate for 3 days at 26°C in an indoor, 50 kl, flow-through, sand-filtered seawater tank exposed to shaded natural light (mean hourly PAR=90±10 μmol photons m^{-2} s^{-1}, maximum PAR=300–350 μmol photons m^{-2} s^{-1}) under a clear ceiling. Twelve nubbins (~2 g $nubbin^{-1}$) were then created from each of the six colonies from each site with pliers for a total of 120 and 24 nubbins reserved for physiological and molecular analyses, respectively. The 72 nubbins from each site were strung on fishing line suspended ~10 cm below the surface, randomly allocated to a position within the acclimation tank and held at the conditions described above for 3 weeks (15 May–7 June) until tissue had covered the site of nubbin fracture.

LBRT

After the 3-week acclimation period, the 72 nubbins from each site were randomly assigned to either a stable temperature (26°C) treatment (*N*=3 tanks, 12 nubbins per tank) or a variable one programmed to fluctuate between 23 and 29°C over a 12 h period (*N*=3 tanks, 12 nubbins per tank). In the latter treatment, temperature was held at 23°C for 5 h, increased to 29°C over the course of 1 h, and then incubated at 29°C for 5 h prior to returning to 23°C over the course of 1 h. Nubbins from each site were incubated in separate tanks to maintain experimental independence, and the temperature in each of the twelve 150-l tanks was controlled by commercial heaters and chillers (AquaTech, Kaohsiung, Taiwan) as described previously (Putnam et al., 2010). All 144 nubbins were suspended on fishing lines 10 cm below the surface to receive similar PAR levels (mean=99 μmol photons m^{-2} s^{-1}) generated by metal halide lamps (150 W), and each tank was characterized by a seawater turnover rate of ~150 l day^{-1}. During the 7 day variable temperature exposure period, which was initiated at 19:00 h on 7 June 2010, HOBO Pendant loggers were used to record the temperature at 10 min intervals in each of the 12 tanks, and a Li-Cor meter was used to document PAR during the light portion of the 12 h:12 h light:dark cycle (lights on 06:00 h, lights off 18:00 h) each day.

Physiological parameters

Prior to experimentation, all coral nubbins to be used for physiological analyses (*N*=120) were buoyantly weighed (*sensu* Spencer-Davies, 1989) in a chamber under an XP105 DeltaRange balance (Mettler-Toledo, Columbus, OH, USA). After 7 days of exposure (15 June 2010) to either the stable or variable temperature regime, F_v/F_m measurements were made on 2 h dark-adapted nubbins with a Diving-PAM underwater fluorometer (Walz, Effeltrich, Germany) as described in another work (Putnam et al., 2010). Nubbins were then re-weighed to calculate growth (normalized to surface area; described below) over the course of the experiment and frozen at –20°C. Upon thawing, 10 nubbins from each tank were blasted with a high-pressure water gun attached to a SCUBA tank to remove tissue, which was decanted into centrifuge tubes and frozen at –20°C after removing aliquots for chl *a* analysis and cell counts.

The tissueless skeleton was dipped twice in molten (65°C) paraffin wax incubated in 200 ml vats within a Thermo-Shandon histological incubation chamber (Waltham, MA, USA) in order to calculate surface area (SA) as described previously (Stimson and Kinzie, 1991). The chl *a* concentration within 1 ml of tissue slurry

was measured in a spectrophotometer after overnight extraction in nine volumes of acetone as in another work (Jeffrey and Humphrey, 1975), normalized to SA and reported as $\mu g\,cm^{-2}$. From another aliquot (100 µl) of tissue slurry, the *Symbiodinium* density of formalin-fixed [4% in 0.1 µm filtered seawater (FSW)] cells was calculated with light microscopy and a hemocytometer (10 counts per sample) as previously described (Fitt et al., 2009). *Symbiodinium* density was also normalized to SA and is reported as $cells\,cm^{-2}$. Areal chl *a* was divided by the *Symbiodinium* density of the same sample to yield $pg\,chl\,a\,cell^{-1}$, which was analyzed and compared across treatments, as described below.

Macromolecular extractions and biological composition parameters

After 7 days of treatment exposure, small (~50 mg) biopsies were fragmented from the two replicates from each tank reserved for molecular analyses, submerged in 500 µl TRI-Reagent (Ambion, Austin, TX, USA) and immediately frozen at –80°C. It should be reiterated that sampling for molecular analyses was conducted on separate nubbins from those used for physiological measurements. This is due to the fact that the buoyant weighing process can require 5–10 min, a period of time in which molecular parameters, such as gene expression, could change. As buoyant weighing occurs at the temperature of treatment, however, it is unlikely that the *Symbiodinium* density and chl *a* content would change over this short period, and so the same nubbins can be used for growth, *Symbiodinium* cell counts and chl *a* analysis.

RNA, DNA and protein were extracted from each of the 24 *S. hystrix* biopsies as in a prior work (Mayfield et al., 2011) with the following exceptions. All centrifugation steps for RNA and DNA purification were performed at 13,000 ***g*** instead of 12,000 ***g***, and nucleic acid pellets were always washed twice with 70% ethanol. Proteins were sonicated gently during the room temperature incubations between spins, as this was shown to better solubilize them in the guanidinium/ethanol wash buffer. RNA, DNA and protein quantity and quality were assessed as described previously (Mayfield et al., 2011). For each sample, total RNA (µg) was divided by total DNA (µg) in order to calculate an RNA:DNA ratio, a proxy for total gene expression. Similarly, a protein:DNA ratio was calculated for each sample as a proxy for total protein expression.

From the DNA phase, *Symbiodinium hsp70* genome copy proportions (GCPs) were calculated with real-time PCR as in a prior work (Mayfield et al., 2011) for normalization of *Symbiodinium* gene expression. Briefly, this strategy is necessary when conducting macromolecular expression analyses with endosymbiotic samples, whose host:*Symbiodinium* biomass ratio may differ between samples, over time or in response to experimental treatments (Mayfield et al., 2009; Mayfield et al., 2010; Mayfield et al., 2011).

For restriction fragment length polymorphism (RFLP) analysis, DNAs were amplified with PCR using the *Symbiodinium 18s* rDNA primers ('ss3z–ss5z'), reagent concentrations and thermocycling conditions of another work (Rowan and Powers, 1991b). The PCR products were purified with the AxyPrep PCR cleanup kit (Axygen, Union City, CA, USA) according to the manufacturer's recommendations, except with a 10 min incubation at 60°C prior to final elution in 20 µl nuclease-free water. The PCR amplicons were then digested with *Taq*I and *Sau3* AI (New England Biolabs, Ipswich, MA, USA) in separate 20 µl reactions as in a prior work (Yang, 2001), and 10 µl of the digested DNAs were electrophoresed on 1.5% Tris acetate EDTA agarose gels, post-stained in an ethidium bromide bath for 20 min and visualized at 610 nm on a Typhoon Trio Scanner (GE Healthcare, Waukesha, WI, USA). The digestion patterns were then compared with those of a prior work (Rowan and Powers, 1991a) to verify *Symbiodinium* identity. Subcladal *Symbiodinium* diversity (*sensu* LaJeunesse, 2002; LaJeunesse et al., 2010; Bellantuono et al., 2011) was not assessed, as the markers used to infer such diversity, particularly the internal transcribed spacer region 2 (ITS2), have issues associated with intragenomic variation (Stat et al., 2009; Stat et al., 2010) that preclude the assignment of a DNA sequence to a single *Symbiodinium* cell (Pochon et al., 2012).

Gene expression

RNA (200 ng) was reverse-transcribed (20 µl reactions) with 1× Solaris RNA spike (Thermo-Scientific) and the High-Capacity cDNA synthesis kit (Applied Biosystems, Foster City, CA, USA), according to the respective manufacturer's recommendations. Assuming equal RNA loading, the Solaris spike controls for differences in reverse transcription (RT) efficiency caused by factors such as enzymatic inhibitors co-purified with the RNA and thereby circumvents the need for a housekeeping gene (Mayfield et al., 2009). Spike-inoculated cDNAs ($2\,\mu l\,reaction^{-1}$) were used as the template in real-time PCRs (20 µl reactions) with the primers found in Table 1, 1× (10 µl) EZ-TIME SYBR Green mastermix with ROX passive reference dye (Yeastern Biotech Co., Taipei, Taiwan), and, in the case of the *pgpase* gene only, 0.5× ($5\,\mu g\,reaction^{-1}$) bovine serum albumin. Real-time PCRs were conducted in an Applied Biosystems 7500 real-time PCR machine, and triplicate reactions of each sample (N=24) were run alongside three serial dilutions of random cDNA samples used to estimate PCR efficiency of each primer set on each 96-well plate (*sensu* Bower et al., 2007).

After a 10 min incubation at 95°C, thermocycling was performed at 95°C for 15 s followed by 60 s at the respective annealing

Table 1. Real-time PCR primers

Gene	Length (bp)	Sequence (5′–3′)	Primer concentration ($nmol\,l^{-1}$)	Annealing temperature (°C)	Cycles	Cellular process	NCBI accession number	Reference
rbcL	126	CAGTGAACGTGGAGGACATGT AGTAGCACGCCTCACCGAAA	200	60	35	Photosynthesis	AAG37859	Present study
psI	136	GTGGAGTTGACATTGACTTGGA TGCTGCTTGGTGGTCTTGTA	500	59	35	Photosynthesis	HM156699	Present study
pgpase	100	TGACAAA CAATTCCACCAAGAG GCTGCAAAGGATGATGAGAAG	250	60	35	Photosynthesis	EU924267	Crawley et al. (2010)
apx1	107	GCCAAGTTCAAGGAGCATGTA AGCTGACCACATCCCAACT	200	60	40	Oxidative stress	HM156698	Present study

The first oligonucleotide listed for each gene represents the forward primer while the second represents the reverse primer.

temperatures for each gene (Table 1). Cycle numbers (Table 1) also varied for each assay, which was always terminated with a melt curve analysis from 65 to 95°C in 10 s increments. Unlike the *Symbiodinium* gene assays, the number of Solaris RNA transcripts reverse transcribed was quantified in each of the 24 cDNA samples using a TaqMan probe-based assay with a proprietary mastermix (including primers) according to the manufacturer's recommendations, and expression of each gene was normalized to the RNA spike recovery as recommended by the manufacturer. Then, the spike-normalized gene expression was divided by the *Symbiodinium* GCP as discussed previously (Mayfield et al., 2011) to control for differential ratios of host:*Symbiodinium* nucleic acids within a complex mixture of biological material. In short, this standardization for RT efficiency and *Symbiodinium* nucleic acid quantity differences between samples is required to generate accurate gene expression data for endosymbiotic, reef-building corals (Mayfield et al., 2009).

Data analysis

All statistical analyses were conducted with JMP (version 5.0, SAS Institute, Cary, NC, USA), and the Shapiro–Wilk test and Levene's test were used to determine whether data sets were normally distributed and of equal variance, respectively (Quinn and Keough, 2002). When either or both conditions were not met, log or square root transformations were conducted prior to statistical tests, and in such cases the back-transformed means were presented in the corresponding figures. Temperature and light data were compared and tested with Student's *t*-tests, and tank effects of physical data were compared with one-way ANOVAs with tank nested within SO and TT. To compare the temperature profiles of the six stable tanks against the six variable temperature tanks, means and standard deviations calculated on each day (N=7) were compared using Student's *t*-tests and one-way nested ANOVAs to determine TT and tank effects, respectively. For the variable TT, temperature data were also partitioned into 'low' (23°C), 'transition' (23–29°C) and 'high' (29°C) groups for a more detailed assessment of SO and tank effects. Two-way nested ANOVAs were used to test for the effects of SO, TT, their interaction and tank nested within TT × SO on both the physiological and molecular parameters. After verifying the absence of a tank effect for each response variable (data not shown), the tank factor was dropped from the model and samples were pooled across tanks, resulting in an N of 30 and 6 for each site and treatment for the physiological and molecular parameters, respectively. Tukey's honestly significant difference (HSD) tests were used to compare individual means. All means are presented ±s.d. unless otherwise indicated.

RESULTS

Field conditions

Temperature was measured at each of the two sites from which the experimental corals were taken, Houwan (non-upwelling site, Fig. 1A,B) and Houbihu (upwelling site, Fig. 1D,E). The mean monthly temperature was similar at each site in 2010: 26.35±1.76°C (N=12) at Houbihu and 26.60±2.03°C at Houwan (Student's *t*-test of monthly means, t=0.32, P=0.75). Importantly, the mean temperature in the 3 months prior to collection (10 February–10 May 2010) was also similar between the two sites (t=0.91, P=0.37) and was 25.75±0.76 and 25.48±1.19°C at Houbihu and Houwan, respectively. In contrast, the temperature variation differed between the two sites (Student's *t*-test of monthly standard deviations, t=2.90, P<0.01); the mean monthly standard deviation over the course of the entire year was 0.99±0.32°C at Houbihu and 0.72±0.04°C (N=12) at Houwan. When looking at the mean monthly standard deviation in temperature in the 3 months prior to experimentation, there was also a significant difference (t=4.69, P=0.01), and the mean monthly standard deviation over this period was 0.93±0.06 and 0.73±0.03°C for Houbihu and Houwan, respectively. The mean monthly range over the course of the entire year was 6.33±2.03 and 3.19±0.61°C for Houbihu and Houwan, respectively, and this difference was also statistically significant (Student's *t*-test of monthly ranges, t=5.13, P<0.01). The mean monthly range in the 3 months prior to coral sampling was 6.15±1.44 and 3.46±0.27°C at Houbihu and Houwan, respectively, and this difference was statistically significant (t=3.90, P=0.02). The largest intra-month fluctuations in temperature at Houbihu were in June (8.4°C) and July (9.6°C), reflecting the periods in which upwelling events were most common. During spring-tide upwelling events at Houbihu in April and May 2010, the average temperature range was ~6°C (23–29°C) per day for up to seven consecutive days (Fig. 1D), and the variable TT used herein (Fig. 1F) aimed to mimic these events. Average diel PAR, based on hourly measurements made between 06:00 and 20:00 h at the approximate depth of coral colony collection (7–8 m) across 3 days, did not differ between sites (Wilcoxon rank-sum test, z=0.66, P=0.51).

Experimental conditions

The mean (±s.e.m.) daily temperature of the Houbihu and Houwan stable tanks (N=3) was 25.88±0.01 and 25.94±0.01°C, respectively, similar to the target of 26°C and the average temperature in the 3 months prior to coral collection and experimentation (~26°C) at each of these sites. Due to low variation between tanks, this small temperature difference was statistically significant (Student's *t*-test, t=7.82, P<0.01), and there was also a statistically significant tank effect (one-way ANOVA of tank nested within treatment, F=49.23, P<0.01), due predominantly to one tank of the Houwan stable temperature group having a significantly lower temperature than that of the other two tanks of that TT and SO (Tukey's HSD, P<0.05).

The variable temperature profile consisted of a 5 h incubation at 23°C, followed by an increase to 29°C over the course of 1 h. Then, samples were incubated at 29°C for 5 h before the temperature was reduced to 23°C over the course of 1 h (Fig. 1F). The mean temperatures of the Houbihu and Houwan variable temperature tanks were 26.40±0.05 and 26.26±0.05°C (N=1010 temperature measurements for each tank), respectively, and this difference was not statistically significant (Student's *t*-test, t=0.47, P=0.64). Furthermore, there were no tank effects (one-way ANOVA of tank nested within SO, F=0.61, P=0.73). However, when partitioning the recorded temperatures into three phases – 'low' (23°C), 'transition' (23–29 and 29–23°C) and 'high' (29°C) for each of the 7 days – statistically significant SO and tank effects were documented. First, the mean (±s.e.m.) low temperature was 23.27±0.02 and 23.11±0.02°C for the three tanks containing corals from Houbihu and Houwan, respectively, and this difference was statistically significant (Wilcoxon rank-sum test, z=3.92, P<0.01). Also, there was a statistically significant effect of tank nested within SO (F=537, P<0.01). Means of 26.66±0.04 and 26.51±0.04°C were recorded at the transition temperatures for the tanks containing corals from Houbihu and Houwan, respectively, and this difference was statistically significant (Student's *t*-test, t=2.47, P=0.02). There was also a statistically significant effect of tank nested within SO (F=3.80, P=0.01). Finally, the high incubation temperatures differed significantly between SO (Wilcoxon rank-sum test, z=2.91, P<0.01) and were 29.28±0.01 and 29.14±0.04°C for the Houbihu and Houwan tanks, respectively. There was a statistically significant effect of tank nested within SO (F=886, P<0.01).

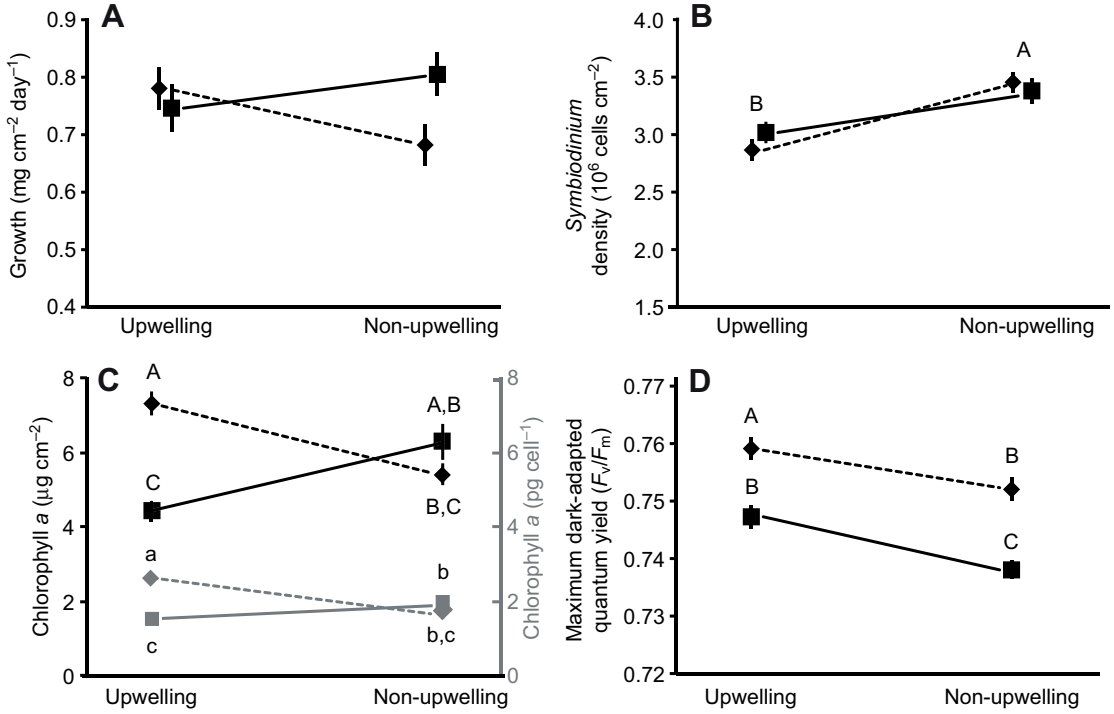

Fig. 2. Physiological parameters. Coral nubbins from the upwelling (Houbihu) and non-upwelling (Houwan) sites were exposed to either a variable (diamonds) or stable (squares) thermal regime (see Fig. 1) for 7 days. (A) Growth of *Seriatopora hystrix* specimens. (B) *Symbiodinium* density. (C) Areal chlorophyll *a* (chl *a*) concentration (black symbols, left-hand *y*-axis) and chl *a* cell^{-1} (gray symbols, right-hand *y*-axis). (D) Maximum dark-adapted quantum yield of photosystem II. Error bars represent ±s.e.m., and, in some cases, do not extend beyond the symbol. Letters adjacent to symbols represent Tukey's honestly significant difference (HSD) groups (α<0.05) and are excluded from A, in which no significant pairwise differences were documented. In B, HSD groups correspond only to site-of-origin differences. The upper and lowercase Tukey's groups shown in C correspond to differences detected within the areal chl *a* and chl *a* cell^{-1} data sets, respectively.

The daily temperature standard deviations for the six stable and six variable tanks across the 7 days of experimentation were found to differ significantly between the two TTs (Wilcoxon rank-sum test, z=7.89, P<0.01), with means of 2.84±0.01 and 0.25±0.01°C for the variable and stable treatments, respectively. There was, however, a tank effect on the average diel standard deviation in temperature (one-way ANOVA of tank nested within treatment, F=28.40, P<0.01) due to one tank of the Houwan stable treatment demonstrating a diel temperature standard deviation that was approximately half that of the other two tanks of that SO and TT (Tukey's HSD, P<0.05). The mean (±s.e.m.) PAR of the 12 tanks (one measurement per tank across each of 7 days) during the light portion of the 12 h:12 h light:dark cycle was 99±1.81 µmol photons m^{-2} s^{-1}, and PAR and did not vary significantly across tanks (one-way ANOVA of tank nested within TT × SO, F=0.24, P=0.86). Furthermore, the PAR was statistically similar (Wilcoxon rank-sum test, z=0.43, P=0.67) between the field sites (mean of both sites=94.3±4.8 µmol photons m^{-2} s^{-1}) and the 12 tanks.

Physiological parameters

A series of physiological parameters were measured in each of the 120 nubbins dedicated for such analyses (Fig. 2). Despite small, though statistically significant, effects of tank on both mean and standard deviation of temperature, physiological and molecular data showed no such tank effects (data not shown), and so data were pooled across tanks, resulting in N=30 and 6 for physiological and molecular parameters, respectively. Growth (Fig. 2A) was not significantly affected by SO or TT but did vary in response to their interaction (Table 2); non-transplanted corals had higher growth rates, although there were no significant pairwise differences (Tukey's HSDs, P>0.05). However, *Symbiodinium* density (Fig. 2B) demonstrated a statistically significant SO response (Table 2), with ~15% higher endosymbiont densities in samples from the non-upwelling site, Houwan. Areal chl *a* (Fig. 2C) was responsive to both TT and the interaction of SO and TT (Table 2), and non-transplanted corals contained significantly higher (~20%) chl *a* cm^{-2}. Also, areal chl *a* was ~1.5-fold higher in corals from the upwelling site exposed to a variable temperature regime relative to samples from this site exposed to stable temperature (Fig. 2C). These changes also held when chl *a* was normalized per cell (Fig. 2C). Finally, both SO and TT significantly affected F_v/F_m (Fig. 2D, Table 2). Specifically, *Symbiodinium* from corals from the upwelling site exhibited ~1% higher F_v/F_m values than those of the non-upwelling site, and *Symbiodinium* from corals exposed to the variable temperature regime displayed ~1% higher values than those incubated at a stable temperature (Fig. 2D).

Biological composition

A variety of RNA, DNA and protein-based parameters were measured in each of two nubbins within each of the 12 tanks. From the DNA phase, a *Symbiodinium hsp70* GCP (Fig. 3A) was calculated with real-time PCR and shown to be statistically similar across sites and treatments (Table 3). Similarly, the RNA/DNA ratio (Fig. 3B) was unresponsive to SO, TT or their interaction (Table 3). In contrast, the protein/DNA ratio (Fig. 3C) demonstrated a significant SO effect (Table 3) and was ~25% higher in samples of the non-upwelling site, Houwan (Fig. 4C). Finally, RFLP-based genotyping (supplementary material Fig. S1) revealed that all 24 samples possessed *Symbiodinium*

Table 2. Results of two-way ANOVAs for physiological data

Source of variation	d.f.	MS	*F*	*P*	Figure
Growth (mg cm^{-2} day^{-1})**					
Site of origin	1	0.00403	0.276	0.601	2A
Temperature treatment	1	0.01966	1.35	0.249	
Site of origin × temperature treatment	1	0.06477	4.43	**0.038**	
Error	119	0.01462	2.02		
Symbiodinium density (cells cm^{-2})					
Site of origin	1	7.69040	30.7	**<0.001**	2B
Temperature treatment	1	0.17680	0.705	0.403	
Site of origin × temperature treatment	1	0.67970	2.71	0.103	
Error	112	0.25080			
Chlorophyll *a* concentration (μg cm^{-2})*					
Site of origin	1	0.00265	0.141	0.708	2C (left *y*-axis)
Temperature treatment	1	0.16729	8.90	**0.004**	
Site of origin × temperature treatment	1	0.57380	30.5	**<0.001**	
Error	116	0.01879			
Chlorophyll *a* concentration (pg cell^{-1})*					
Site of origin	1	0.69410	10.1	**0.002**	2C (right *y*-axis)
Temperature treatment	1	1.30600	19.0	**<0.001**	
Site of origin × temperature treatment	1	3.36300	49.0	**<0.001**	
Error	113	0.06865			
Maximum dark-adapted quantum yield (F_v/F_m)					
Site of origin	1	0.00200	18.3	**<0.001**	2D
Temperature treatment	1	0.00502	45.8	**<0.001**	
Site of origin × temperature treatment	1	0.00003	0.310	0.578	
Error	116	0.00017			

Log- and square root-transformed data are denoted by * and **, respectively, and statistically significant results (P<0.05) are highlighted in bold. All calculations were performed with JMP as described in the Materials and methods.

of clade C based on results from both *Taq*I and *Sau3* AI digests of the *18s* rDNA gene. Previous work in Southern Taiwan (R. Gates, unpublished data) has found that the same *Symbiodinium* ITS2 types (C1, C3 and C59) were associated with *S. hystrix* at both Houbihu and Houwan. While the intragenomic variation associated with the ITS2 marker does not allow for a current assessment of the degree to which *Symbiodinium* diversity may have driven the changes in physiological response documented herein, it is hoped that new molecular markers for *Symbiodinium* (Pochon et al., 2012) and functional work across a variety of *Symbiodinium* types (*sensu* Sampayo et al., 2007; Stat et al., 2008) will allow for future elucidation of this critically important topic in our field.

Gene expression

High-quality holobiont RNAs inoculated with exogenous spikes were reverse transcribed to cDNA and used as a template in real-time PCRs for *Symbiodinium* gene expression analyses. There was a statistically significant interaction effect on the recovery of the Solaris RNA spike (Table 3), suggesting that the RT reaction was not equally efficient across the 24 samples. After normalizing mRNA expression of the *Symbiodinium* candidate genes to both Solaris spike recovery and the *Symbiodinium hsp70* GCP, it was found that *rbcL* expression (Fig. 4A) differed significantly in response to TT, with ~2-fold more transcripts in specimens of the variable TT (Fig. 4A). *psI* (subunit III) expression (Fig. 4B) also differed in response to TT, with ~1.9-fold more transcripts measured in samples of the variable TT. Unlike *rbcL*, *psI* expression also was significantly influenced by SO (Table 3), with 1.5-fold higher expression in *Symbiodinium* from corals from the upwelling site. TT also significantly affected expression of *pgpase* (Fig. 4C, Table 3), which was expressed at 1.6-fold-higher levels in corals exposed to variable temperature. However, none of the factors or their interaction had a significant effect on the expression of *apx1* (Fig. 4D, Table 3).

DISCUSSION

Interaction effects on coral physiology: higher growth rates and chl *a* content in non-transplanted corals

Thermal history has been shown to influence the capacity of reef-building corals to acclimate to elevated temperatures (e.g. D'Croz and Mate, 2004; Castillo and Helmuth, 2005; Middlebrook et al., 2008; Edmunds, 2009), though this topic is still quite poorly understood, particularly from a molecular perspective. Previous studies on this topic have had few consistent conclusions, though some researchers found that prior exposure to stressful conditions could lead to enhanced ability to tolerate later stressors (Brown et al., 2002a; Brown et al., 2002b; Oliver and Palumbi, 2011; Thompson and van Woesik, 2009; Guest et al., 2012). These works led us to hypothesize that corals would show an interaction effect in response to the LBRT, whereby corals from an upwelling environment would demonstrate enhanced physiological performance upon exposure to a variable temperature regime, while corals from a more stable thermal environment would perform relatively better under the stable temperature regime. In fact, this observation was only supported by growth and chl *a* content, both of which were higher in non-transplanted controls (Fig. 2). Specifically, corals from the upwelling site possessed higher chl *a* content and exhibited higher growth rates when exposed to the fluctuating temperature regime, while those specimens from the non-upwelling site exhibited higher chl *a* content and growth rates at the stable temperature regime.

The authors of a similar study (Smith et al., 2007) conducted a reciprocal transplant *in situ,* whereby corals were transplanted between a low-energy backreef and a high-energy forereef, and observed faster linear extension in non-transplanted controls. As such, both the coral specimens of this prior work and those sampled herein demonstrated phenotypic plasticity, though they were ultimately well adapted to the environment from which they were

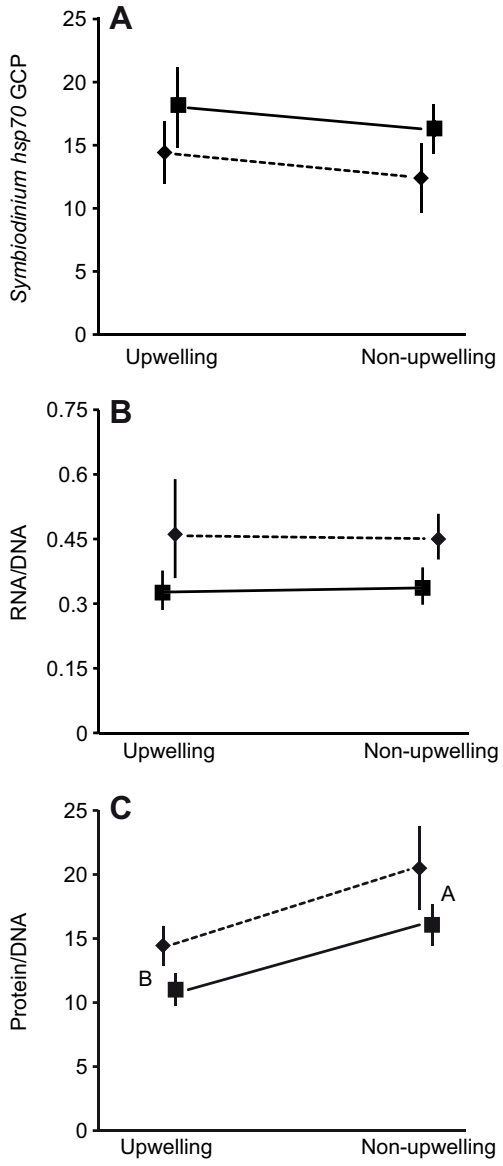

Fig. 3. Biological composition parameters. *Symbiodinium* heat shock protein-70 (*hsp70*) genome copy proportions (GCPs; A), RNA/DNA ratios (B) and protein/DNA ratios (C) were measured in *Seriatopora hystrix* specimens from Houbihu (upwelling site) and Houwan (non-upwelling site) exposed to either variable (diamonds) or stable (squares) temperature regimes for 7 days. Error bars represent ±s.e.m. Letters adjacent to symbols in C represent Tukey's HSD groups (α<0.05) and signify only site-of-origin effects.

initially sampled. However, as discussed in greater detail below, while growth is arguably the most easily interpretable measure of coral fitness (Hughes, 1987), data derived from cellular and sub-cellular responses are required to develop the physiological mechanisms by which the coral acclimation to altered temperature regimes documented herein occurred.

SO effects

Higher *Symbiodinium* density and protein/DNA ratios in corals from the non-upwelling site

Had only growth and chl *a* content been measured, the overarching hypothesis proposed herein, as well as those proposed by researchers in coral biology (e.g. Brown et al., 2002b) and other fields (Cunningham and Read, 2003; Stillman, 2003; Somero, 2010), would have been substantiated; corals perform better at a familiar temperature regime. However, as a wider range of biological scales was examined herein, a closer look at the additional parameters measured (Table 4) reveals that, in fact, the physiological response of corals to variable temperature may be quite complex. For instance, certain traits, such as the protein/DNA ratio (Fig. 3C) and *Symbiodinium* density (Fig. 2B), showed adherence to SO and were unaffected by TT or the SO × TT interaction; specifically, corals from the non-upwelling site were shown to possess higher *Symbiodinium* densities and protein:DNA ratios at the termination of the experiment. Interestingly, another study (D'Croz and Mate, 2004) also documented higher *Symbiodinium* densities and total protein in corals sampled from a non-upwelling reef relative to those from an upwelling reef. It is likely that corals with higher *Symbiodinium* densities would have higher protein content, as the *Symbiodinium* occupy nearly the entire volume of the coral gastrodermal tissues (Chen et al., 2012) and are highly proteinaceous (Weston et al., 2012). In fact, our prior work (Mayfield et al., 2011) has shown that the protein:DNA ratio was variable over time in *S. hystrix*, and we suggested that such variation could indeed be due to differences in *Symbiodinium* density. Both the spatial differences in *Symbiodinium* density and holobiont protein documented herein and previously (D'Croz and Mate, 2004) and the temporal differences in these parameters documented in prior studies (Mayfield et al., 2010; Mayfield et al., 2011) highlight the importance of normalizing *Symbiodinium* macromolecular expression in a way that accommodates variation in the host:*Symbiodinium* biomass ratio.

It is unclear why corals from reefs characterized by decreased thermal heterogeneity would possess higher densities of *Symbiodinium*. It is possible that corals from upwelling reefs possess lower *Symbiodinium* densities due to the potential for *Symbiodinium* to generate ROS in response to rapidly increasing temperatures (Lesser, 1997). mRNA levels of *apx1*, whose respective protein degrades certain ROS species into less harmful intermediates, was detected at similar levels between SOs (Fig. 4D). Although this may indicate that ROS levels were similar between corals of the two sites, a direct analysis of the concentration of ROS species, as well as expression of additional molecules involved in ROS detoxification [e.g. catalase (Lesser and Shick, 1990)], is warranted to conclusively determine the role of ROS in driving *Symbiodinium* density differences. As such, it remains to be demonstrated whether relatively lower densities of *Symbiodinium* are characteristic of upwelling reefs and whether or not such an adaptation is due to a correlation between *Symbiodinium* density and ROS production under thermally variable temperatures. Finally, as a second explanation for these findings, Houwan is characterized by significantly higher nutrient levels than Houbihu (Liu et al., 2012), and these relatively higher nitrogen levels, in particular, may allow for a higher standing stock of *Symbiodinium*, which are thought to be nitrogen-limited in many corals and sea anemones (Wang and Douglas, 1998).

Higher F_v/F_m and *psI* mRNA expression in samples from the upwelling site

In addition to *Symbiodinium* density and the protein:DNA ratio, both F_v/F_m and *Symbiodinium psI* (subunit III) mRNA expression were also characterized by significant SO effects (Table 4), and were, specifically, higher in samples from the upwelling site, Houbihu. F_v/F_m is a common index used by coral biologists to infer the efficiency of the initial stages of electron transport in PSII within dark-adapted *Symbiodinium* (e.g. Warner et al., 1996). Although a statistically significant 1% increase in F_v/F_m was found in corals

Table 3. Results of two-way ANOVAs for biological composition and *Symbiodinium* gene expression data

Source of variation	d.f.	MS	*F*	*P*	Figure
Symbiodinium hsp70 genome copy proportion (GCP)					
Site of origin	1	0.00186	0.485	0.495	3A
Temperature treatment	1	0.00755	1.97	0.177	
Site of origin × temperature treatment	1	0.00002	0.005	0.946	
Error	21	0.00383	0.819		
RNA/DNA ratio*					
Site of origin	1	0.00025	0.002	0.967	3B
Temperature treatment	1	0.5382	3.80	0.067	
Site of origin × temperature treatment	1	0.00351	0.025	0.877	
Error	21	0.1416	1.28		
Protein/DNA ratio*					
Site of origin	1	0.1289	8.65	**0.009**	3C
Temperature treatment	1	0.06590	4.38	0.051	
Site of origin × temperature treatment	1	0.00088	0.059	0.811	
Error	21	0.01490			
Solaris RNA spike					
Site of origin	1	4.721	0.742	0.402	Not shown
Temperature treatment	1	1.364	0.214	0.650	
Site of origin × temperature treatment	1	100.1	15.7	**0.001**	
Error	19	6.363	5.45		
Symbiodinium rbcL mRNA expression**					
Site of origin	1	0.9107	0.195	0.664	4A
Temperature treatment	1	20.98	4.48	**0.048**	
Site of origin × temperature treatment	1	0.2120	0.045	0.834	
Error	21	4.680	1.58		
Symbiodinium psI (subunit III) mRNA expression*					
Site of origin	1	0.2039	4.76	**0.043**	4B
Temperature treatment	1	0.3446	8.04	**0.011**	
Site of origin × temperature treatment	1	0.02492	0.581	0.456	
Error	21	0.04286	4.12		
Symbiodinium pgpase mRNA expression**					
Site of origin	1	0.09879	0.167	0.687	4C
Temperature treatment	1	3.526	5.97	**0.025**	
Site of origin × temperature treatment	1	0.07728	0.131	0.722	
Error	21	0.5903	2.10		
Symbiodinium apx1 mRNA expression*					
Site of origin	1	0.00763	0.123	0.730	4D
Temperature treatment	1	0.1982	3.19	0.091	
Site of origin × temperature treatment	1	0.06896	1.11	0.306	
Error	21	0.06215	1.50		

Statistically significant results (P<0.05) are highlighted in bold, and log- and square root-transformed data are denoted by * and **, respectively. All calculations were performed with JMP as described in the Materials and methods.

from the upwelling site relative to those of the non-upwelling site, it is unclear whether such a small change would have implications for coral photosynthesis and health. It is tempting, though, to speculate whether there is a biologically significant correlation between photosystem gene expression and F_v/F_m; it may be that cells with more efficient photosystems could accommodate higher electron loads and thus require higher levels of photosystem gene and protein expression.

TT effects: higher F_v/F_m and PTG mRNA expression upon exposure to a variable temperature regime

F_v/F_m and *psI* mRNA expression were also affected by TT, and, in addition to expression of *pgpase* and *rbcL*, were found to be at higher levels in corals exposed to a temperature regime that varied from 23 to 29°C over a 12 h period. In other words, both F_v/F_m and expression of the three PTGs were higher in samples exposed to a variable temperature regime for 7 days. Interestingly, our prior work (Putnam et al., 2010) also documented higher F_v/F_m in samples exposed to fluctuating temperatures in comparison to those incubated at stable temperatures, though in that study, only corals from the upwelling site, Houbihu, were used, and so this result was not unexpected. However, we also documented negative effects on coral physiology when the fluctuating treatment encompassed temperatures of 30–32°C (Putnam and Edmunds, 2009; Putnam and Edmunds, 2011), which have long been known to elicit the coral thermal stress response (Brown, 1997; Fitt et al., 2001). As temperatures used in the present study were below levels known to evoke stress in this species (Hung et al., 1998; Mayfield et al., 2011), it does not seem as if the coral specimens exposed to the variable temperature regime were physiologically compromised. In fact, an argument will be made below that they had an elevated photosynthetic capacity based on a variety of indices.

Unlike *psI* gene expression and F_v/F_m, which varied in response to both SO and TT, but not their interaction, the *rbcL* and *pgpase* genes appeared to be more strongly influenced by temperature directly and not the thermal history of the corals. *Symbiodinium rbcL* has been molecularly (Rowan et al., 1996) and biochemically characterized (Lilley et al., 2010), and a recent study found RBCL protein expression to be decreased in response to elevated temperature (Doo et al., 2012); although the latter was conducted

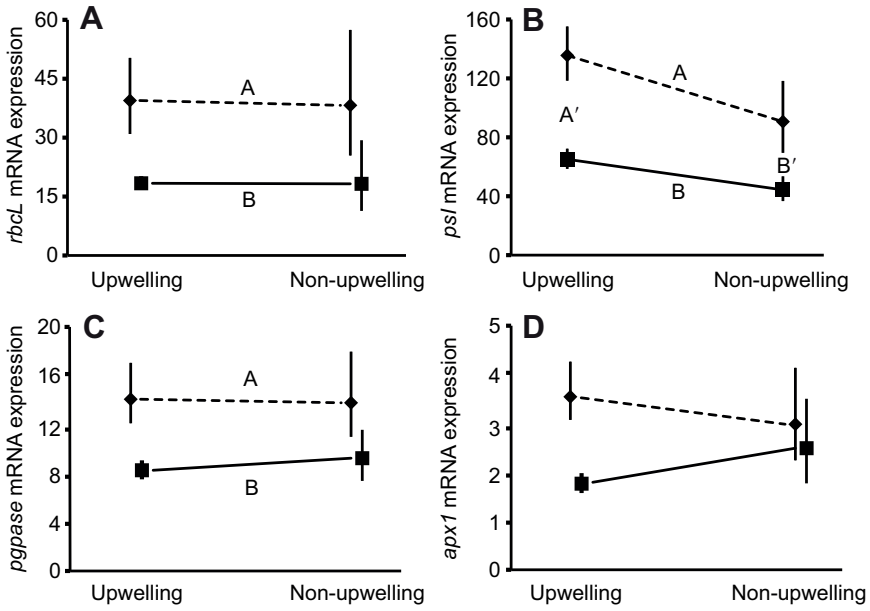

Fig. 4. *Symbiodinium* gene expression. *Symbiodinium* ribulose-1,5-bisphosphate carboxylase/oxygenase (*rbcL*; A), photosystem I (psI, subunit III; B) phosphoglycolate phosphatase (*pgpase*; C) and ascorbate peroxidase (*apx1*; D) gene expression were normalized to Solaris spike recovery and real-time PCR-derived *Symbiodinium* genome copy proportions (GCPs) in *Seriatopora hystrix* specimens from Houbihu (upwelling site) and Houwan (non-upwelling site) exposed to either variable (diamonds) or stable (squares) temperature regimes for 7 days. Error bars represent ±s.e.m., and, in some cases, do not extend beyond the icon. Letters adjacent to icons represent Tukey's HSD groups (α<0.05) and signify significant temperature treatment (TT) effects in A and C. In B, uppercase letters represent Tukey's TT HSD groups, whereas uppercase letters with primes (A′ and B′) represent Tukey's site-of-origin HSD groups.

with a foram–diatom symbiosis, it is possible that the strong temperature effect on *rbcL*/RBCL expression is a ubiquitous phenomenon of marine microalgae (Leggat et al., 2004). Importantly, these substantial changes in both *rbcL* and *pgpase* gene expression between temperature regimes, regardless of SO, point to a capacity for plasticity at the molecular level in this widely distributed Indo-Pacific reef coral that could have implications for carbon fixation.

The fact that *psI*, *rbcL* and *pgpase* gene expression were relatively elevated in samples of the variable TT suggests that an increase in carbon fixation could indeed have occurred in these samples. First, elevated *psI* expression could suggest an enhanced capacity for electron transport and capture (Varotto et al., 2000), which would increase the number of ATP and NADPH molecules available for Calvin cycle enzymes. Such a circumstance might have driven the relative increase in *rbcL* expression in these same samples. *rbcL* encodes the rate-limiting enzyme of the Calvin cycle, RBCL, a protein known to be particularly inefficient (Whitney and Yellowlees, 1995). As such, increased levels of RBCL expression could allow for carbon fixation to occur at a faster rate and increase the potential for autotrophy in the coral holobiont. Secondly, increased expression of *pgpase*/PGPase could be necessary when both the light and dark reactions are occurring at high rates due to the enzyme's role in metabolizing negative regulators of the carbon fixation pathways (Kaplan et al., 1991; Suzuki, 1995). Thus, in contrast to the hypothesis that elevated *pgpase* expression would be indicative of photorespiration stemming from thermal-stress-derived photoinhibition, it is now argued that its upregulation instead could lead to higher capacity for Calvin cycle enzyme function. Ultimately, to determine whether such increases in *Symbiodinium* PTG expression actually lead to increases in carbon fixation, as has been documented in higher plants (Mayfield et al., 1995; Murchie et al., 2005) and phytoplankton (Paul and Pichard, 1998), and presumed to occur in corals (Crawley et al., 2010), future studies should simultaneously measure both PTG expression as was conducted herein and the degree of *Symbiodinium* carbon fixation and translocation to the host with radiolabeling-based approaches (*sensu* Furla et al., 2000; Cantin et al., 2009).

Methodological quality control

Numerous studies have attempted to use molecular tools to document the coral response to environmental changes (e.g. DeSalvo et al., 2008; Bay et al., 2009; Császár et al., 2009; Reyes-Bermudez et al., 2009; Voolstra et al., 2009; DeSalvo et al., 2010; Portune et al., 2010; Starcevic et al., 2010; Hoogenboom et al., 2011; Kenkel et al., 2011; Leggat et al., 2011; Levy et al., 2011), generating a wealth of potentially interesting findings with regard to the molecular capacity of corals to acclimate to changes they may face over the

Table 4. Summary of results from the *Seriatopora hystrix* laboratory-based reciprocal transplant study

Parameter	SO effect	TT effect	Interaction effect	Major finding(s)
Growth			✓	Faster growth in non-transplanted corals
Symbiodinium density	✓			Non-upwelling site > upwelling site
Chlorophyll *a* (chl *a*) concentration		✓	✓	Variable temperature > stable temperature Higher chl *a* in non-transplanted corals
Maximum dark-adapted quantum yield of photosystem II (F_v/F_m)	✓	✓		Upwelling site > non-upwelling site Variable temperature > stable temperature
Protein/DNA ratio	✓			Non-upwelling site > upwelling site
Symbiodinium rbcL expression		✓		Variable temperature > stable temperature
Symbiodinium psI expression	✓	✓		Upwelling site > non-upwelling site Variable temperature > stable temperature
Symbiodinium pgpase expression		✓		Variable temperature > stable temperature

Site of origin (SO), temperature treatment (TT) and interaction effects were deemed statistically significant at P<0.05 and, in such cases, are denoted by a tick. Only response variables for which significant differences were detected have been included..

coming century. However, only one prior study has documented the sub-cellular response of corals to environmental changes with molecular methods that accommodate the endosymbiotic nature of coral tissues (Mayfield et al., 2011). In fact, in order to generate biologically meaningful macromolecular expression data for endosymbiotic organisms, such as reef-building corals, it is essential to employ not only commonplace methodological controls, such as exogenous spikes to ensure that RT efficiency, for instance, is similar between samples, but also biological composition controls (i.e. *Symbiodinium* GCPs) to ensure that differences in the host:*Symbiodinium* biomass ratio do not bias gene expression data. For instance, decreased levels of *Symbiodinium* gene expression may be measured in a bleached coral relative to a healthy coral if appropriate biological composition controls, such as those utilized herein, are not taken, simply because of the former possessing lower densities of *Symbiodinium* relative to the latter. Therefore, we further advocate the use of biological composition controls for all works seeking to document macromolecular expression in endosymbiotic organisms.

Conclusions

This represents the first reciprocal transplant study of corals that employs techniques that allow for the generation of biologically meaningful data with respect to the molecular biology of these environmentally sensitive organisms, and it is hoped that future studies will utilize similar approaches to investigate the sub-cellular changes of reef corals exposed to, for instance, global climate change simulations. We used real-time PCR-based gene expression, as well as other physiological and molecular approaches hypothesized to gauge the coral physiological response, and the ensuing data set suggests that corals may indeed perform well when incubated under fluctuating temperatures, possibly *via* an enhanced capacity for photosynthesis and carbon fixation. Ultimately, though, corals grew faster at conditions consistent with their thermal history (Podrabsky and Somero, 2004), as hypothesized, and growth is arguably the most indicative sign of organismal fitness. Collectively, then, it appears that both environmental history and temperature regime are important for predicting the physiological response of reef corals, and future transcriptome (cDNA) sequencing work of the samples used herein with Illumina Tru-Seq (San Diego, CA, USA) technology and a Genome Analyzer IIx will help to better uncover the molecular mechanisms through which such acclimation occurred.

LIST OF ABBREVIATIONS

apx1/APX1	ascorbate peroxidase
chl *a*	chlorophyll *a*
FSW	filtered seawater
F_v/F_m	maximum dark-adapted quantum yield
GCP	genome copy proportion
HSD	honestly significant difference
hsp70/HSP70	heat shock protein-70
ITS2	internal transcribed spacer region 2
LBRT	laboratory-based reciprocal transplant
PAR	photosynthetically active radiation
pgpase/PGPASE	phosphoglycolate phosphatase
psI/PSI	photosystem I
PSII	photosystem II
PTG	photosynthesis-targeted gene
rbcL/RBCL	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit
RFLP	restriction fragment length polymorphism
ROS	reactive oxygen species
RT	reverse transcription
SA	surface area
SO	site of origin
TT	temperature treatment

ACKNOWLEDGEMENTS

Steve Doo is acknowledged for his assistance with sample processing and comments on earlier versions of the manuscript. Dr Peter Edmunds is also thanked for valuable discussions on experimental design. Dr Yi-Yuong Hsiao is graciously thanked for sharing of laboratory space, within which the majority of the molecular analyses were conducted. Finally, we thank Dr Ruth D. Gates for her contribution of unpublished *Symbiodinium* nucleic acid sequence data. This is Hawaii Institute of Marine Biology contribution no. 1524 and the School of Ocean and Earth Science and Technology (SOEST) manuscript no. 8775.

FUNDING

A.B.M. was funded by an international postdoctoral research fellowship from the National Science Foundation (NSF) of the United States of America (OISE-0852960), as well as an NSF East Asia and Pacific Summer Institutes (EAPSI) fellowship. *The Journal of Experimental Biology* provided funding to A.B.M. through a travel fellowship, and both the PADI Foundation and PADI Project Aware contributed funds to A.B.M. for conducting of laboratory analyses. Funds from the International Society for Reef Studies/Ocean Conservancy (ISRS/TOC) and the United States Environmental Protection Agency (FP917199) were awarded to H.M.P. Finally, intramural grants from NMMBA to T.-Y.F. funded the LBRT experiment, as well as the physiological analyses.

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