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The effects of temperature on gene expression in the Indo-Pacific reef-building coral *Seriatopora hystrix*: insight from aquarium studies in Southern Taiwan

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Abstract Corals from upwelling reefs of Southern Taiwan have been shown to acclimate to both highly variable and abnormally elevated temperatures. To better understand the genetic basis for Taiwanese reef coral acclimation to such temperature regimes, eight and six genes hypothesized to be thermo-sensitive were targeted in specimens of the common stony coral *Seriatopora hystrix* and their endosymbiotic dinoflagellate (genus *Symbiodinium*) communities, respectively, from two reefs in Southern Taiwan exposed to two different temperature profiles. Coral specimens from Houbihu, a reef characterized by extensive, spring-tide upwelling, displayed few provocative gene expression changes when exposed for two days to a temperature they rarely encounter *in situ*, 30℃. In a second experiment, corals from Houbihu and Houwan, the latter being a reef characterized by more stable temperatures, were exposed to either a stable (26℃) or a fluctuating (23-29℃ over a 5-h period) temperature regime for seven days, and it was found that 50% of the genes demonstrated significant changes in expression across treatments, regardless of the site of origin. This suggests that exposure to a variable temperature for seven days may have a more dramatic effect on the sub-cellular behavior of this species than a two-day incubation at a stable, though potentially stress-inducing, temperature.

Keywords Acclimation; Acclimatization; Coral reef, Dinoflagellate; Endosymbiosis; Gene expression; Temperature

Background

Given the threats of global climate change (GCC) towards scleractinian corals and the reef ecosystems they construct, there is an urgent need to better understand their physiological (Gates and Edmunds, 1999) and sub-cellular (van Oppen and Gates, 2006) mechanisms of acclimatization to altered temperature regimes (Mayfield et al., 2013a). An emerging model for the study of GCC effects on reef corals is *Seriatopora hystrix* (Figure 1), which is amongst the most ubiquitously distributed scleractinians in the Indo-Pacific (Loh et al., 2001; Veron, 2000). *S. hystrix* has served as a model coral for research in diverse locations across the Pacific Rim, such as Australia (e.g., Bongaerts et al., 2011) and Taiwan (e.g., Fan et al., 2006; Putnam et al., 2010; Tung et al., 2006), and is amongst the most sensitive corals to environmental change (Loya et al., 2001). However, in Southern Taiwan, *S. hystrix* lives in relatively warm waters (summer mean = ~28.5℃) whose temperatures can change rapidly due not only to the tidal cycle (Meng et al., 2008), but also to episodic upwelling (Chen et al., 2004; Jan and Chen, 2008).

S. hystrix is known to have limited dispersal capacity (Ayre and Hughes, 2000), a trait that may be associated with its ability to readily adapt to a diverse array of environments (Nunes et al., 2009; Sanford and Kelly, 2011). Indeed, prior work on *S. hystrix* populations from the thermally dynamic upwelling sites mentioned above found that neither the coral hosts nor their resident *Symbiodinium* populations mount a gene-level molecular chaperone (heat shock protein-70 [*hsp70*]) response when exposed to 30℃ for 48 h (Mayfield et al., 2011; i.e., the elevated temperature experiment [ETE]). This was hypothesized

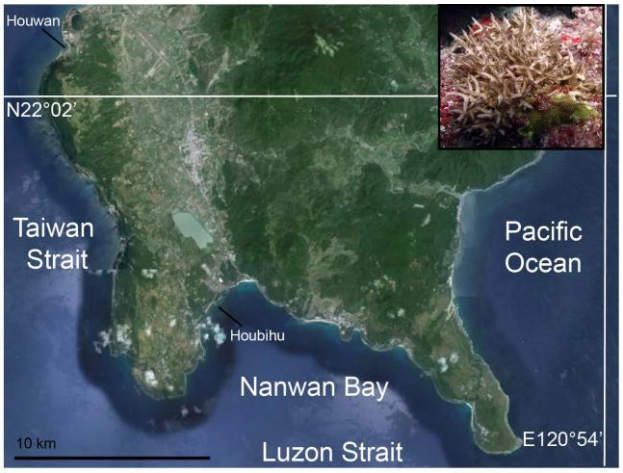

Figure 1 Map of Southern Taiwan's Hengchun Peninsula, including the locations of the two study sites (Houwan and Houbihu), alongside an image of the target organism *Seriatopora hystrix*, which is common at both sites. For a detailed treatise on the temperature environment of the two sites, readers are pointed towards Mayfield et al. (2012a). Briefly, the average monthly temperature ranges of Houbihu and Houwan from 2009 to 2010 were 6.33±2.03 and 3.19±0.61℃, respectively, and this represents a significant difference (student's *t*-test, t=5.13, p<0.01). The size of the *S. hystrix* colony in the inset is approximately 15 cm in diameter.

to be a stress-inducing temperature given that it is ~1℃ greater than the average summer temperature of Houbihu (Figure 1), the upwelling site (UWS) within Nanwan Bay (Taiwan's southernmost embayment) from which the experimental corals were collected; briefly, previous studies (e.g., Coles and Brown, 2003; Hoegh-Guldberg and Smith, 1989) have found that extended exposure to temperatures >1℃ above the mean summer high can result in bleaching in many reef coral species.

Given these results, Mayfield et al. (2011) hypothesized that *S. hystrix* residing in the UWS may have special adaptations for life in thermodynamically variable environments, as the temperature at the UWS can change by up to 9℃ in a single day during spring-tide upwelling events that occur throughout the year (but mainly in the northern summer; Mayfield et al., 2012a). Mayfield et al. (2012a, 2013c) attempted to test this hypothesis with a laboratory-based reciprocal transplant in which corals from this UWS were exposed to either a variable (23-29℃ over a 5-h period) or stable (26℃) temperature treatment while conspecifics from a non-upwelling site (i.e., NUWS) characterized by relatively stable (over diel and annual timescales) temperatures, Houwan (Figure 1), were exposed to the same two temperature regimes for seven days. It was hypothesized that corals transplanted to a "foreign" temperature regime would be physiologically compromised in this variable temperature experiment (VTE), though this hypothesis was confirmed only upon assessment of coral growth (Mayfield et al., 2012a); other parameters appear to have been more significantly influenced by the temperature regime alone.

To gain more insight into the genetic basis underlying the phenotypic plasticity of these Taiwanese *S. hystrix* populations, expression of 14 gene mRNAs whose respective proteins and cellular pathways have been hypothesized to be disrupted upon exposure to elevated temperature was measured in samples of the ETE and VTE (Table 1); these included mRNAs encoding proteins involved in photosynthesis, metabolism, osmoregulation, the cytoskeleton, and the stress response (see the Materials and Methods for detailed rationale for choosing the respective genes.). Gene expression was hypothesized to remain similar over time and between treatments in samples from the UWS exposed to 30℃ for two days in the ETE given the lack of *hsp70* modulation documented at these temperatures by Mayfield et al. (2011). In contrast, it was hypothesized that the majority of the genes would be expressed at different levels between the stable and variable temperature treatments in the VTE in corals from the UWS only, given that exposure to a familiar temperature change can drive significant changes in mRNA expression in other marine organisms (e.g., Gracey et al., 2008). It was further hypothesized that corals from the UWS would demonstrate higher levels of target gene expression than conspecifics from the NUWS. Briefly, corals inhabiting variable temperature environments could be expected to express higher mRNA levels; the reasoning behind this is due to the need for rapid increases in protein translation at times at which temperature increases have incapacitated the standing pool of intracellular proteins in response to elevated temperature-induced protein denaturation (Hazel and Prosser, 1974). This is a strategy employed by not only intertidal limpets (Dong et al., 2008), but also other corals (Barshis et al., 2013).

Table 1. Summary of the two *Seriatopora hystrix* experiments conducted at Taiwan's National Museum of Marine Biology and Aquarium (NMMBA). Although the experiments were conducted a year apart, the same aquarium system was used to conduct each one. Also, the acclimation temperatures, which aimed to approximate *in situ* values at the time of coral collection, were slightly different for the two experiments due to *in situ* differences at the time of coral collection.

Experiment	Elevated temperature experiment	Variable temperature experiment
Abbreviation	ETE	VTE
Manuscript(s)	Mayfield et al. (2011)	Mayfield et al. (2012a, 2013c)
Source population(s) (see Fig. 1.)	Houbihu (upwelling site) only	Houbihu (upwelling site) Houwan (non-upwelling site)
Time of coral collection *in situ*	June 2009	May 2010
Specimen morphology	whole colony	experimentally fragmented nubbin
Acclimation time	3 weeks	3 weeks
Acclimation temperature	27℃	26℃
Acclimation PAR[1]	300-400 μmol m^{-2} s^{-1}	300-400 μmol m^{-2} s^{-1}
Time of experimentation	July 2009	June 2010
Temperature treatments	control (27℃) high (30℃)	stable (26℃) variable (23-29℃ over a 5-hr period)
Experimental period PAR	90±10 μmol m^{-2} s^{-1}	90±10 μmol m^{-2} s^{-1}
Length of treatment	48 h	7 d
Biological replicates/number of aquaria	3 aquaria/treatment x 2 treatments = 6 aquaria total	3 aquaria/treatment/site of origin (SO) x 2 treatments x 2 SO = 12 aquaria total
Technical replicates	3/tank in Mayfield et al. (2011) 1/tank herein	2/tank
Sampling times	0, 6, 12, 24, and 48 h (time 0 samples not discussed herein)	0 and 7 d (time 0 samples not discussed herein)
Sample size (*n*)	90 in Mayfield et al. (2011) 24 herein (see text for details.)	24 (time 7 d only) in Mayfield et al. (2012a) 24 (time 7 d only) herein
Number of gene mRNAs targeted	2 in Mayfield et al. (2011) + 12 herein = 14 total	4 in Mayfield et al. (2012a) + 10 herein = 14 total

Notes: [1]photosynthetically active radiation.

1 Results

1.1 ETE: *Symbiodinium* gene expression

Recovery of the Solaris™ spike (Thermo-Scientific) was similar between treatments and over time (data not shown) in samples of the ETE (Table A1), all 24 of which were found to be composed only of *Symbiodinium* of clade C (C_t<30; C_t>35 for clade A and D assays). *Symbiodinium* gene expression was normalized to both spike recovery and the *Symbiodinium* genome copy proportion (GCP; Figure 2A); the latter of which was also found to be similar across treatments and time (Table A1). Expression of ribulose-1,5-bisphosphate carboxylase-oxygenase large subunit (*rbcL*; Figure 2B), photosystem I (subunit III; *psI*; Figure 2C), phosphoglycolate phosphatase (*pgpase*; Figure 2D), nitrate transporter-2 (*nrt2*; Figure 2E), and ascorbate peroxidase (*apx1*; Figure 2F) was similar over time and between treatments (Table A1).

1.2 ETE: host coral gene expression

Expression of the eight host genes was normalized to recovery of the Solaris spike (data not shown) and the host GCP (Figure 3A); the latter of which was found to be stable over time and between treatments (Table A2). Only β-actin (*actb*; Figure 3B) and α-tubulin (*tuba*; Figure 3D) were significantly affected by the interaction of time and temperature; expression of the former in the control nubbins increased ~4-fold from the 24 to the 48 h sampling time (Figure 3B). In contrast, *tuba* was expressed at 2-fold higher levels in

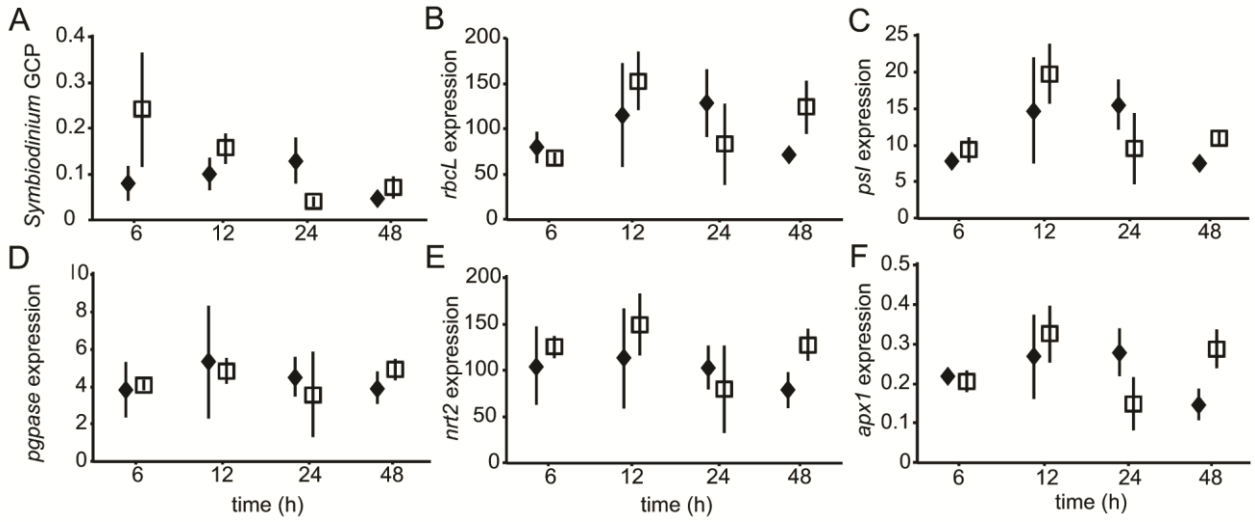

Figure 2 ETE: *Symbiodinium* gene expression. *Symbiodinium* genome copy proportion (GCP [unit-less]; A) and expression of five genes (B-F) in *Seriatopora hystrix* samples exposed to either a control (27℃; hollow squares) or elevated (30℃; filled diamonds) temperature for 48 h. Please see the text or Tables A1-3 for full gene names. Gene expression data were normalized as described in the text and presented as unit-less. In all panels, error bars represent standard error of the mean (n=3 replicates per treatment-time).

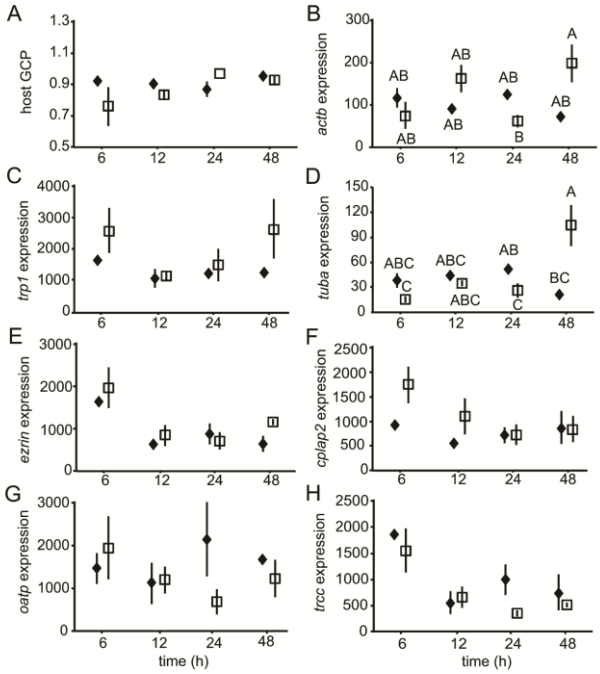

Figure 3 ETE: host coral gene expression. Host genome copy proportion (GCP [unit-less]; A) and expression of seven target genes (B-H) in *Seriatopora hystrix* samples exposed to either a control (27℃; hollow squares) or elevated (30℃; filled diamonds) temperature for 48 h. Please see the text or Tables A1-3 for full gene names. Gene expression data were normalized as described in the text and presented as unit-less. In all panels, error bars represent standard error of the mean (n=3 replicates per treatment-time). In panels B and D, letters adjacent to icons signify Tukey's honestly significant difference groups (p<0.05) for the interaction effect of time and temperature. Expression of host coral heat shock protein-70 (*hsp70*) gene was measured by Mayfield et al. (2011).

the high temperature samples of time 24 relative to the controls. Then, at sampling time 48, expression of this gene was 5-fold higher in controls. Tropomyosin (*trp1*; Figure 3C), *ezrin* (Figure 3E), phospholipase-α2 (*cplap2*; Figure 3F), organic anion transporter (*oatp*; Figure 3G), and transient receptor cation channel (*trcc*; Figure 3H) were, on the other hand, expressed at similar levels between treatments across the four sampling times (Table A2).

1.3 VTE: *Symbiodinium* gene expression

Expression of *nrt2* (Figure 4A) and heat shock protein-70 (*hsp70*; Figure 4B) was assessed in *Symbiodinium* populations within the 24 samples, all of which were shown to be of clade C only by real-time PCR (C_t<30; C_t>35 for clade A and D assays). Although there were no significant effects of temperature, site of origin, or their interaction on expression of the former (Table A3), expression of *Symbiodinium hsp70* was significantly higher (1.8-fold) in samples exposed to a variable temperature regime (Table A3). The influence of the temperature regime had a significantly greater influence on *Symbiodinium* gene expression in samples of the VTE (4/6 of the target genes were significantly affected by temperature when combining the results herein with the three genes [*psI*, *pgpase*, and *rbcL*] assessed by Mayfield et al. [2012a].) versus those of the ETE (0/6; X^2=7.6, p<0.01).

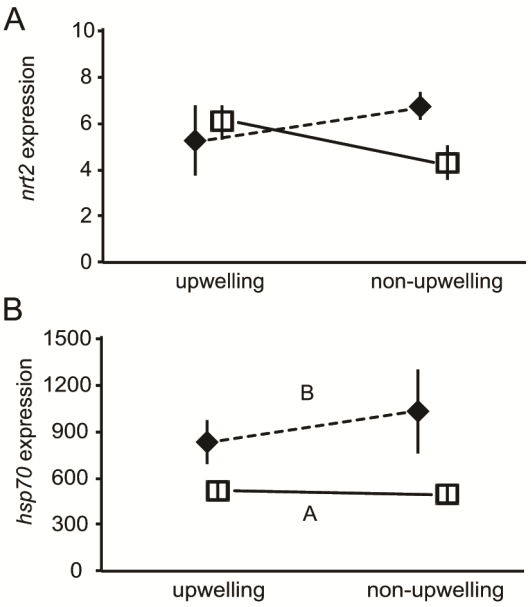

Figure 4 VTE: *Symbiodinium* gene expression. *nrt2* (A) and *hsp70* (B) gene expression from *Symbiodinium* within *Seriatopora hystrix* nubbins from Houbihu ("upwelling" site [UWS]) or Houwan ("non-upwelling" site [NUWS]) exposed to either a stable (hollow squares) or variable (filled diamonds) temperature treatment for seven days. Please see the text or Tables A1-3 for full gene names. Gene expression data were normalized as described in the text and presented as unit-less. Error bars represent standard error of the mean in all panels (n=3 replicates [6 pseudo-replicates] per treatment-site of origin), and in panel B only, the letters adjacent to icons denote Tukey's honestly significant difference groups (p<0.05) for the temperature effect only.

1.4 VTE: host coral gene expression

Expression of the stress-targeted gene *hsp70* (Figure 5A), four cytoskeleton-targeted genes; *actb* (Figure 5C), *trp1* (Figure 5D), *tuba* (Figure 5E), and *ezrin* (Figure 5F), and three osmoregulation-targeted genes; *cplap2* (Figure 5G), *oatp* (Figure 5H), and *trcc* (Figure 5I), were normalized to both recovery of the Solaris spike (data not shown) and the host GCP; the latter of which was shown to be stable between sites of origin and temperature treatments (data not shown).

Although host *hsp70* expression was found at significantly higher (~3.5-fold) levels in samples exposed to the stable temperature regime (Figure 5A), there was neither site of origin nor interaction effects (Table A3). There was also no significant correlation between *Symbiodinium* and host *hsp70* expression (Figure 5B; linear regression t-test, t=2.1, p>0.05).

The expression of both *actb* (Figure 5C) and *cplap2* (Figure 5G) was affected by the experimental treatments (Table A3); the former was found to be 30% higher in samples exposed to the variable temperature regime. *cplap2* was characterized by an interaction effect in which corals from the NUWS exposed to the variable temperature regime expressed 2.1-fold higher levels than corals from the UWS exposed to this same temperature profile. Of the 14 target genes assessed across both compartments, 2 (host *actb* and *tuba*; 14%) were significantly affected by temperature in the ETE, whereas 7 (*Symbiodinium rbcL*, *psI*, *pgpase* [from Mayfield et al., 2012a], and *hsp70* and host coral *hsp70*, *actb*, and *cplap2*; 50%) demonstrated significant differences between temperature regimes in the VTE. This difference in the overall proportion of target genes demonstrating a significant difference across the two experiments was marginally significant (X^2=3.9, p=0.05).

1.5 VTE: host coral genotyping

With the exception of Sh4-001, all microsatellite loci were polymorphic (Table 2); the former was excluded from the calculations. The number of alleles (N_A), observed heterozygosity (H_o), and expected heterozygosity (H_e) for each remaining locus ranged from 1 to 7 (mean=3.9), 0 to 0.83 (mean=0.53), and 0 to 0.89 (mean=0.63), respectively. Deviations from Hardy-Weinberg equilibrium and heterozygote deficiencies were suggested by inbreeding coefficient (F_{IS}) values, which were significant for samples from the UWS (F_{IS}=0.24, p<0.05), but not the NUWS (F_{IS}=0.099, p=0.20). The overall F_{IS} value of 0.16 was statistically significant (p<0.05), suggesting that inbreeding may exist within these two populations. There were no null alleles across the seven loci (data not shown). Finally, no significant genetic differentiation was found between the Houbihu and Houwan samples (fixation index [F_{ST}]=-0.012, p>0.05), though this could be partially attributed to the small sample size (n=6 for each site of origin).

Table 2 Host coral genotyping results. "N" = sample size. "N_A" = number of alleles. "N_{PA}" = number of private alleles. "H_o" = observed heterozygosity. "H_e" = expected heterozygosity. "F_{IS}" = inbreeding coefficient. "UWS" = upwelling site (Houbihu). "NUWS" = non-upwelling site (Houwan).

Locus		UWS	NUWS	Allele size range (base pairs)
	N	6	6	
Sh4-001	N_A	2	1	126-184 (UWS), 184 (NUWS)
	N_{PA}	1	0	
	H_o	0.17	0	
	H_e	0.17	0	
	F_{IS}	0.00	NA	
Sh2.15	N_A	3	4	268-277 (UWS), 268-278 (NUWS)
	N_{PA}	0	1	
	H_o	0.17	0.50	
	H_e	0.71	0.71	
	F_{IS}	0.78*	0.32	
Sh2-002	N_A	4	5	123-146 (UWS), 123-146 (NUWS)
	N_{PA}	1	2	
	H_o	0.50	0.83	
	H_e	0.77	0.76	
	F_{IS}	0.38	-0.11	
Sh4.28	N_A	5	4	106-119 (UWS), 107-117 (NUWS)
	N_{PA}	2	1	
	H_o	0.67	0.67	
	H_e	0.74	0.64	
	F_{IS}	0.11	-0.053	
Sh2-006	N_A	3	3	105-114 (UWS), 105-110 (NUWS)
	N_{PA}	1	1	
	H_o	0.50	0.50	
	H_e	0.44	0.44	
	F_{IS}	-0.15	-0.15	
Sh4.24	N_A	6	7	260-315 (UWS), 259-313 (NUWS)
	N_{PA}	4	5	
	H_o	0.83	0.50	
	H_e	0.88	0.89	
	F_{IS}	0.057	0.46*	
Sh3-004	N_A	3	2	148-154 (UWS), 148-151 (NUWS)
	N_{PA}	1	0	
	H_o	0.50	0.50	
	H_e	0.59	0.41	
	F_{IS}	0.17	-0.25	
Overall				Mean
	N_A	3.7	4.2	3.9
	N_{PA}	0.48	0.58	0.53
	H_o	0.62	0.64	0.63
	H_e	0.24*	0.099	0.16*

Notes: *****p<0.05.

1.6 Site of origin comparisons

Upon pooling data across experiments (Figure 6), the *Symbiodinium* GCP (data not shown) was found to be similar between sites of origin (student's *t*-test, t=0.91, p=0.37). On the other hand, *rbcL* expression was significantly higher (16-fold) in samples from the UWS relative to those of the NUWS (Wilcoxon Z=3.5, p<0.001). Although expression of *psI* was statistically similar between the two sites (Z=1.3, p=0.19), *pgpase* was expressed at 2.0-fold higher levels in corals of the UWS (Z=4.7, p<0.001). In contrast, *nrt2* was expressed at similar levels across sites (student's t=0.74, p=0.46), as was *apx1* (Wilcoxon Z=1.8, p=0.076). Finally, *Symbiodinium hsp70* was expressed at significantly higher (4.1-fold) levels in corals from the NUWS relative to those of the UWS (Z=3.6, p<0.001).

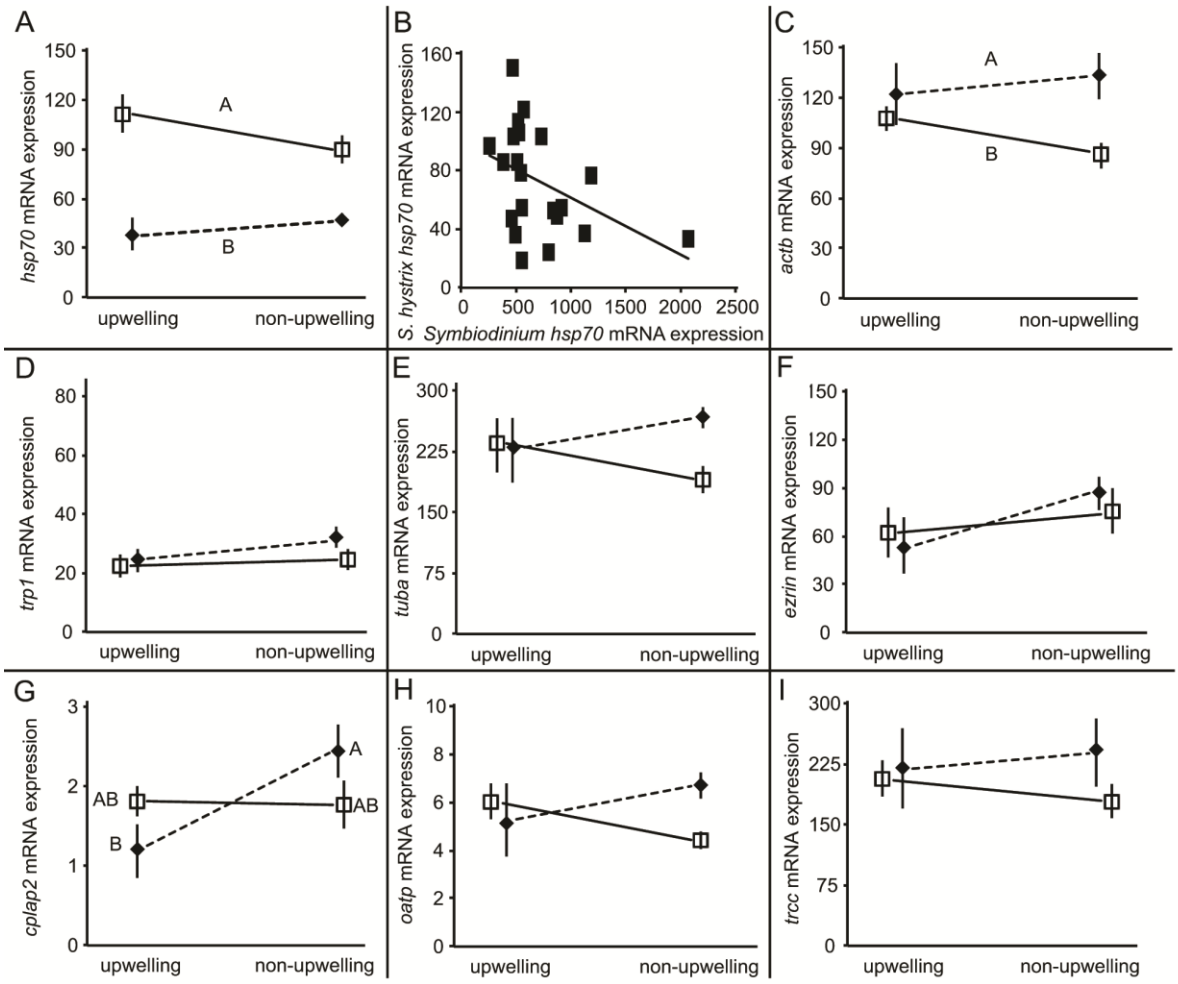

Figure 5 VTE: host coral gene expression. Expression of eight host coral genes; *hsp70* (A), *actb* (C), *trp1* (D), *tuba* (E), *ezrin* (F), *cplap2* (G), *oatp* (H), and *trcc* (I), and correlations in *hsp70* expression within the holobiont (B) in *Seriatopora hystrix* nubbins from Houbihu ("upwelling" site [UWS]) or Houwan ("non-upwelling" site [NUWS]) exposed to either a stable (hollow squares of panels A and C-I) or variable (filled diamonds of panels A and C-I) temperature for seven days. Please see the text or Tables A1-3 for full gene names. Gene expression data were normalized as described in the text and presented as unit-less. Error bars represent standard error of the mean (n=3 replicates [6 pseudo-replicates] per treatment-site of origin). In panels A and C, letters adjacent to icons correspond to Tukey's honestly significant difference (HSD) temperature treatment groups only (p<0.05), while letters adjacent to icons in panel G correspond to the interaction effect of site of origin and temperature treatment (Tukey's HSD, p<0.05).

With respect to the host coral genes, *actb* was expressed at similar levels between sites (Wilcoxon Z=0.20, p=0.84). On the other hand, *trp1* was expressed at 41-fold higher levels in corals of the UWS (Z=3.0, p<0.01), and *tuba* was expressed at 2.3-fold higher levels in those from the NUWS (Z=3.5, p<0.001). *ezrin* and *cplap2* were expressed at significantly higher (9.4 and 320-fold, respectively) levels in samples from the UWS relative to those from the NUWS (Z=2.6 and 2.8, p=0.01 and <0.01, respectively), as was *oatp* (140-fold difference; Z=2.3, p<0.05, though see caveats in caption for Figure 6).

The osmoregulation gene *trcc* was expressed at 3.1-fold higher levels in corals from the UWS (Z=2.3, p<0.05). Finally, the host molecular chaperone *hsp70* was expressed at significantly lower (1.8-fold) levels in corals from the UWS relative to those of the NUWS (Wilcoxon Z=2.5, p<0.05). Although 7/14 (50%) of the genes were more highly expressed in samples from the UWS versus only 3/14 (21%) of the genes demonstrating higher expression in samples from the NUWS, this difference in proportions was not statistically significant (X^2=2.5, p=0.11).

2 Discussion

From a collective assessment of the data obtained herein and in prior works (Table 3), it appears that *S. hystrix* colonies from Houbihu (UWS), Southern Taiwan do not demonstrate a pronounced mRNA-level response to a temperature hypothesized to induce stress, 30℃. Specifically, expression of none of the six *Symbiodinium* genes spanning three cellular processes;

Table 3. **Summary of results.** No coral colonies of the elevated temperature experiment (ETE) or nubbins of the variable temperature experiment (VTE) died over the course of the experiments, and for the ETE, in which only a small proportion of the 18 colonies was sacrificed for molecular analyses, the remaining colonies survived under ambient conditions at NMMBA for at least six months after the termination of the experiment.

			ETE			VTE		
Physiology			temperature	time	interaction	temperature	site of origin	interaction
survival								
growth (mg cm^{-2} day^{-1})			NA					*
Symbiodinium density (cells cm^{-2})			NA				*	
chlorophyll a concentration (pg $cell^{-1}$)			NA			*		*
maximum quantum yield of PSII (F_V/F_M)			NA			*	*	
Molecular composition								
Symbiodinium hsp70 GCP				*				
RNA/DNA ratio								
protein/DNA ratio				*			*	
Symbiodinium genotype								
host coral genotype			NA					
Gene expression								
Gene	**Compartment**	**Function**						
Solaris spike recovery		exogenous spike						*
rbcL	*Symbiodinium*	photosynthesis				*		
psI	*Symbiodinium*	photosynthesis				*	*	
pgpase	*Symbiodinium*	photosynthesis				*		
nrt2	*Symbiodinium*	metabolism						
apx1	*Symbiodinium*	stress response						
hsp70	*Symbiodinium*	stress response		*		*		
hsp70	host	stress response		*		*		
actb	host	cytoskeleton			*	*		
trp1	host	cytoskeleton						
tuba	host	cytoskeleton			*			
ezrin	host	cytoskeleton						
cplap2	host	osmoregulation						*
oatp	host	osmoregulation						
trcc	host	osmoregulation						

Note: *$p<0.05$. "NA" = not assessed.

photosynthesis, metabolism, and the stress response, differed between treatments at any sampling time in the ETE. On the other hand, expression of host *tuba* decreased 2-fold from the 24 to the 48 h sampling time in the high temperature samples. Although such a decrease could provide evidence in support of the hypothesis that the coral cytoskeleton may become compromised under potentially stress-inducing conditions (Mayfield and Gates, 2007), a similar change in only one other target gene was observed; *actb* was also expressed at higher levels in controls relative to high temperature samples at the final sampling time, though this difference was not statistically significant. It should be noted that *actb*

was the only target gene that was affected by temperature in both experiments, discounting its utility as a housekeeping gene for this coral species. However, the difference in expression between samples of the two temperature regimes was only ~30% in the VTE.

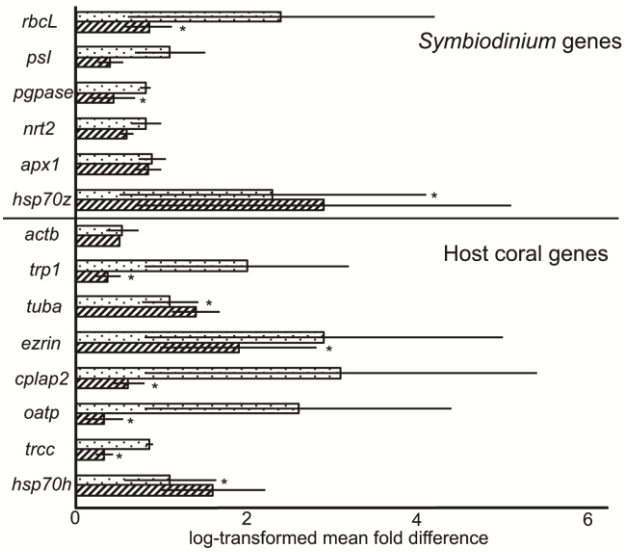

Figure 6 Global site of origin differences in gene expression. To compare the average expression of each of the 14 target genes across the two sites of origin, Houbihu (upwelling site [UWS]; speckled columns) and Houwan (non-upwelling site [NUWS]; hatched columns), data from the 12 UWS samples of the *Seriatopora hystrix* variable temperature experiment (VTE) and all 24 samples of the elevated temperature experiment (ETE) were compared against the expression data from the 12 NUWS samples from the VTE. Please see the text or Tables A1-3 for full gene names. Gene expression values were first converted to fold changes relative to the lowest expression sample within the 48 assessed. Then, mean fold changes were log-transformed in order to present all data on the same scale. Asterisks ("*") denote significant differences detected by Bonferroni-adjusted Wilcoxon rank-sum tests, and error bars represent standard error of the mean (n=36 and 12 for the UWS and NUWS, respectively). The statistically significant difference in host coral *oatp* expression between sites must be interpreted with caution, as different primer concentrations were used between experiments (Table 4). "*hsp70z*" and "*hsp70h*" refer to the *hsp70* homolog from the *Symbiodinium* and host coral compartments, respectively.

This absence of a temperature-driven response in 12/14 target genes across both compartments of the holobiont in samples exposed to 30℃ may suggest that exposure to a stable, elevated temperature regime does not elicit a stress response in specimens of *S. hystrix* from Southern Taiwan. Exposure to 30℃ has typically been shown to result in stress in *S. hystrix* elsewhere (Hoegh-Guldberg and Smith, 1989; Loya et al., 2001), and this should manifest at the gene level over the course of only several hours (Feder, 1996). Although it is tempting to speculate that this lack of an mRNA-level response to exposure to 30℃ in none of the six *Symbiodinium* target genes, and all but two (*actb* and *tuba*) of the eight host coral gene targets, stems from adaptations to life in a fluctuating temperature environment (Mayfield et al., 2011), a targeting of a greater number of gene and protein candidates over a longer sampling time will be necessary to demonstrate that these corals indeed display no signs of a sub-cellular stress response upon exposure to this temperature; indeed, <0.1% of the transcriptome of the *S. hystrix*-*Symbiodinium* holobiont was queried herein, and current efforts employing next generation sequencing-based technology (Mayfield et al., in prep.) seek to assess the whole-transcriptome response of these samples to gain further insight into the molecular mechanisms by which *S. hystrix* acclimates to altered temperature regimes in the laboratory and acclimatizes to them in the upwelling reef ecosystems in which they reside.

Additionally, in order to determine whether the absence of a gene-level is related to thermal history, a static ETE would also need to be conducted with conspecifics from the NUWS; such an experiment would help to strengthen the hypothesis put forth in prior works (e.g., Oliver and Palumbi, 2011; Mayfield et al., 2013b) that corals from variable temperature environments perform better at elevated temperatures than those from stable temperature habitats. If such a differential response were indeed documented, it would be attributable to acclimatization rather than adaptation, as the UWS and NUWS populations were found to be genetically identical (Table 2) and possess similarly homogenous *Symbiodinium* assemblages. That said, neutral markers, such as the microsatellites employed herein, cannot detect genetic differences driven by environmental changes (Foret et al., 2007) and so future studies seeking to uncover genetic differences underlying phenotypic plasticity should not only utilize larger sample sizes but also analyze genes that could be hypothesized to be under selection.

In contrast to incubation at a static, elevated temperature for two days, exposure to a variable temperature regime for seven days had a dramatic influence on gene expression; 50% of the genes were significantly affected by temperature in the VTE. This was mainly driven by the *Symbiodinium* gene expression profiles; although no *Symbiodinium* genes were significantly influenced by a 2-day exposure to 30℃, four of the six targets (67%); *rbcL*, *psI*, *pgpase* (assessed in Mayfield et al., 2012a), and *hsp70* (assessed herein), were significantly influenced by temperature regime in the VTE. This was a significantly higher proportion, suggesting that exposure to a variable temperature regime over seven days had a more dramatic influence on the *Symbiodinium* mRNA-level response than exposure to a static, elevated one for two days. Mayfield et al. (2012a) took the elevated F_V/F_M values in these same samples to indicate that corals exposed to variable temperature may have a high capacity for photosynthesis, though an analysis of the degree of carbon fixation (*sensu* Furla et al., 2000) will be necessary to demonstrate whether such mRNA concentration and F_V/F_M increases actually lead to higher photosynthetic output of the resident clade C *Symbiodinium* populations.

The reason why exposure to a familiar, variable temperature regime had such a strong effect on *Symbiodinium* gene expression may be due to the need to rapidly adjust levels of protein translation in unstable environments. It has recently been found that corals may "front-load" gene mRNAs (Mayfield et al., 2011; Barshis et al., 2013), whereby expression is constitutively elevated at relatively high levels due to potentially high protein translation demands stemming from life in unstable environments. This strategy is commonly employed by intertidal invertebrates (Somero, 2010), such as limpets (Dong et al., 2008), and could explain the high levels of photosynthesis gene expression in the *Symbiodinium* populations within samples exposed to a variable temperature regime. mRNA front-loading theoretically allows for faster rates of protein translation that may be necessary when the standing pool of proteins becomes denatured due to abrupt temperature changes, such as the dramatic thermal shifts associated with upwelling events (Hochachka and Somero, 2002). By maintaining high levels of mRNA expression during such periods, corals and their resident *Symbiodinium* populations would have a higher scope for translation of proteins that may have been compromised by dramatic increases or decreases in temperature. This front-loading phenomenon may also explain why 7 of the 14 target genes were expressed at significantly higher levels in corals from the UWS, Houbihu, while only 3 were expressed at higher levels in those of the NUWS, Houwan (Figure 6), a site characterized by a considerably more stable temperature profile (Mayfield et al., 2012a).

On the other hand, two of the three stress genes targeted herein and in a prior work (Mayfield et al., 2011), *Symbiodinium* and host coral *hsp70*, were instead expressed at higher levels in corals from the NUWS, suggesting that corals of the UWS do not necessarily front-load stress gene expression to a greater extent than conspecifics from the NUWS. As such, the front-loading mRNA loading phenomenon may be utilized for certain cellular response pathways, such as photosynthesis, but not all. A more thorough understanding of the behavior and function of transcription factors involved in the thermal acclimation response, such as heat shock factors (HSFs, Akerfelt et al., 2010), will ultimately aid in the development of an understanding of why the mRNA-level response differed between corals exposed to familiar or unfamiliar temperature regimes, as well as between sites. Such an analysis may also help to elucidate whether thermal history (e.g., prior exposure to upwelling) or the temperature regime itself is more important in driving the sub-cellular response of *S. hystrix* to temperature changes.

It is currently unclear whether such differences in gene expression between sites and temperature regimes documented herein and in previous works (e.g., Barshis et al., 2013) actually lead to an enhanced capacity for protein translation, as expression of the respective proteins was not measured in previous coral-based studies, nor was their activity. As such, it is hoped that both gene and protein expression, as well as activity of the latter, will be simultaneously measured in coral samples of future temperature manipulation studies such that the understanding of the molecular means by which corals respond to differential temperature regimes can be better

developed. A greater knowledge of the sub-cellular basis of coral phenotypic plasticity, as well as their acclimatization to altered environments, will allow for the establishment of hypotheses as to their response to the increases in temperature and pCO_2 that are predicted to characterize the oceans in the coming decades as a result of GCC (Ogawa et al., 2013).

3 Materials and Methods

3.1 Experiments

3.1.1 ETE

The details of both the elevated (i.e., ETE; Mayfield et al., 2011) and variable temperature (i.e., VTE; Mayfield et al., 2012a) experiments are described in the respective manuscripts and summarized in Table 1. Briefly, specimens were collected only from the UWS, Houbihu, in the former. This coral reef (Figure 1) is characterized by dramatic temperature fluctuations as a consequence of spring-tide upwelling events (Mayfield et al., 2012a); indeed, changes of up to 9℃ can occur over the course of a single day in the summer months. Triplicate tanks containing three *S. hystrix* colonies collected from the UWS were incubated at either the control (27℃) or elevated (30℃) temperature for 48 h, and ~50-mg branches (~2 mm radius x ~7 mm length = ~100 mm^2 surface area) were taken from each of the 18 colonies after 0, 6, 12, 24, and 48 h (*n*=90 samples). An effort was made to avoid sampling from the same section of the colony multiple times in order to ensure that sampling stress did not bias results. Data from the time 0 samples are not presented herein.

3.1.2 VTE

In the VTE, coral nubbins fragmented from colonies originally from the UWS (Houbihu) and the NUWS (Houwan) were exposed to either a stable temperature treatment (26℃, *n*=3 tanks for corals of each site of origin) or one that fluctuated from 23 to 29℃ over a 5-h period (*n*=3 tanks for corals of each site of origin). Houwan, which is a small bay (Figure 1) located approximately 15 km north of Houbihu on the western side of the Hengchun Peninsula, does not experience upwelling events, and temperature rarely fluctuates by more than 1℃ on any given day of the year (Mayfield et al., 2012a). In this study, two nubbins from each of the 12 tanks were sampled at the beginning of the experiment, as well as after seven days of treatment exposure; however, only the latter 24 samples were analyzed for molecular response variables.

3.2 RNA, DNA, and protein extractions

RNA, DNA, and protein were extracted in sequential fashion from each of the 90 and 24 samples of the ETE and VTE, respectively. Briefly, RNA was reverse transcribed to complementary DNA (cDNA) in both studies, and the cDNAs were used as template in real-time PCRs for target gene analysis. As the reverse transcription input RNA amounts and, with one exception, real-time PCR primer concentrations were identical for both studies, gene expression results could be directly compared for all but one gene (discussed in Table 4). The DNAs were used for normalization of gene expression data (*sensu* Mayfield et al., 2009), *Symbiodinium* genotyping (*sensu* Correa et al., 2009), and host genotyping (*sensu* Underwood et al., 2006; VTE samples only); all of these procedures are described in detail below. The proteins were quantified as described previously (Mayfield et al., 2011) and used to calculate a protein/DNA ratio, which serves as a proxy for total protein expression.

3.2.1 ETE: extractions

Prior to cDNA synthesis (200 ng RNA/reaction), RNAs from the three pseudo-replicated samples from each of the six tanks were mixed, re-extracted with the GeneMark® Plant RNA Miniprep purification kit (Hopegen Biotechnology) according to the manufacturer's recommendations (including DNase treatment), and the 24 samples (*n*=3 for each treatment at each sampling time) from times 6, 12, 24, and 48 h were re-quantified and assessed for quality as in Mayfield et al. (2012b). In a similar manner, the triplicate DNAs from each tank and time were pooled and re-purified with the AxyPrep™ PCR cleanup kit (Axygen; manufacturer's recommendations) prior to their use in real-time PCRs (20 ng/reaction), and the 24 DNAs from corals sampled after 6, 12, 24, and 48 h (*n*=3 for each treatment-sampling time) were analyzed.

3.2.2 VTE: extractions

RNA and DNA were re-extracted from coral tissues that had been frozen in 500 μl TRI-Reagent® (Life Technologies) at -80℃ as described in Mayfield et al. (2012a), and the duplicate, pseudo-replicated RNAs and DNAs within each tank were left un-pooled prior to cDNA synthesis (described below) and real-time PCRs, respectively.

Table 4. **PCR primers, mastermix composition, and thermocycling conditions**. When two values are given under the primer concentration ("nM primer"), annealing temperature, or PCR cycles ("Cycle #") columns, they correspond to values used in the *Seriatopora hystrix* elevated (i.e., ETE) and variable (i.e., VTE) temperature experiments, respectively. When only one value appears, the same conditions were used for samples of both experiments. Full genes names have been written out in the text and Tables A1-3.

Gene	Length (bp) [a]	Sequence ('5-3')	nM primer	Annealing temp. (℃)	Cycle #	Cellular process	NCBI accession	Reference
Solaris spike	NA	TGCAAAGCCAATTCCCGAAG	125	63	36	exogenous spike	NA	Putnam et al., 2013
		CCATTGTAGTGAACAGTAGGAC			34			
***SYMBIODINIUM* TARGET GENES**								
rbcL	126	CAGTGAACGTGGAGGACATGT	200 [b]	60.5	32	photosynthesis	AAG37859	Mayfield et al., 2012a
		AGTAGCACGCCTCACCGAAA						
psI	136	GTGGAGTTGACATTGACTTGGA	500 [b]	59	39	photosynthesis	HM156699	Mayfield et al., 2012a
		TGCTGCTTGGTGGTCTTGTA						
pgpase	100	TGACAAACAATTCCACCAAGAG	250 [b]	60	32	photosynthesis	EU924267	Crawley et al., 2010
		GCTGCAAAGGATGATGAGAAG						
nrt2	97	CCACCCATTTCAGGACCTAT	200	61.5	33	metabolism	HM147134	Mayfield et al., 2013a
		CCAGGGACCTAGCAAACAA		62	38			
hsp70	86	CTGTCCATGGGCCTGGAGACT	500 [c]	62.5	40	stress response	EU476880	Mayfield et al., 2009
		GTGAACGTCTGTGCCTTCTTGGTT						
apx1	107	GCCAAGTTCAAGGAGCATGTA	150 [b]	61	40	stress response	HM156698	Mayfield et al., 2012a
		AGCTGACCACATCCCAACT						
***SERIATOPORA HYSTRIX* TARGET GENES**								
actb	100	GACTCGTATGTCGGTGATGAAGCT	50	60	29	cytoskeleton	HM147127	Mayfield et al., 2010
		TTTCCATGTCATCCCAGTTGGT						
trp1	108	GCTGCAAAGATCGCTAACCTG	50	61.5	33	cytoskeleton	HM147128	Mayfield et al., 2010
		TCCAACCAAGGTGACCTCTTC						
tuba	110	ACATCTGTCGGCGCAACCT	50	61.5	33	cytoskeleton	HM147129	Mayfield et al., 2010
		GCACCATCAAAGCGCAGTGA						
ezrin	162	CAGCGGAAAATGCTTCAGC	400	59	35	cytoskeleton	HM147133	present study
		TTCATCAACCCTCTGCTTAGTG						
cplap2	174	GCAAACGGACGGAATCAAAGA	100	60	35	osmoregulation	HM156700	present study
		GGCATCTTCAAACACGGAGA						
oatp	130	ACGGTTGGCTGTTTGACACT	250	60	35	osmoregulation	HM147131	present study
		AACACATGGCAAGTTCCGG	200					
trcc	114	TGGTCGGTTTGGCTGTTG	250	60	30	osmoregulation	HM147132	present study
		TCCATTGTGGGAGAGATTCTTC						
hsp70	62	AATCCAGGAGTTGCTCAGAAATT	500 [c]	59	28	stress response	AB201749	Mayfield et al., 2011
		TCTGACACAGCTTCACTCTTA						

Note: [a] "bp" = base pairs. [b] ETE only; VTE assays were conducted by Mayfield et al. (2012a). [c] VTE only; ETE assays were conducted by Mayfield et al. (2011).

3.3 Target gene selection

In the *Symbiodinium* compartment, three cellular processes previously suggested to be thermo-sensitive (Mayfield et al., 2013b, d) were targeted; photosynthesis, metabolism, and the stress response. Regarding the former, three photosynthesis-targeted genes; *rbcL*, *psI* (subunit III), and *pgpase* were targeted with real-time PCR (Table 4), as *Symbiodinium* have repeatedly been shown to be photoinhibited when exposed to foreign temperature regimes (Warner et al., 1996; 1999). Such photoinhibition has been found to manifest at the mRNA level (Crawley et al., 2010). Furthermore, protein expression of RBCL, which is the rate-limiting enzyme of the Calvin Cycle, was previously found to decrease in *Symbiodinium* within coral larvae (Putnam et al., 2013), as well as diatom symbionts within foraminiferers (Doo et al., 2012), exposed to elevated temperatures.

One metabolism-targeted gene, *nrt2*, was additionally assayed in samples of both studies (Table 4). Briefly, expression of this gene, whose respective protein may be involved in the translocation of nitrate within the holobiont, was previously hypothesized to be thermo-sensitive (Mayfield et al., 2013a) given that the nutrient dialogue between *Symbiodinium* and the host coral could be impaired during periods of elevated temperature (Mayfield and Gates, 2007). Finally, expression of two stress-targeted genes, *hsp70* and *apx1* (Table 4), was measured in the *Symbiodinium* compartment. The latter is involved in the detoxification of reactive oxygen species (ROS), which have been documented to be produced in *Symbiodinium* within thermally-challenged corals (Lesser 1996; 1997; 2006). The expression of all six *Symbiodinium* target genes was hypothesized to be similar between temperature treatments and over time in the ETE, yet vary between temperature treatments in the VTE.

Eight genes across three cellular processes- the cytoskeleton (n=4), osmoregulation (n=3), and the stress response (n=1)- were targeted in the coral host. Regarding the former two processes, cell volume control and the cytoskeleton have been hypothesized to be significantly compromised by changes in the abiotic environment of a coral (Mayfield and Gates, 2007). The reason underlying this hypothesis is because the host coral must theoretically spend a considerable amount of energy to osmoregulate since *Symbiodinium* occupy the majority of the volume of its gastrodermal cells (Chen et al., 2012). As *Symbiodinium* are photoinhibited at stress-inducing conditions (Jones et al., 1998), the consequent decrease in their translocation of compatible solutes, such as glycerol, into the host cytoplasm could cause a drop in osmotic pressure and a consequent collapse of the cytoskeleton. In fact, a temporary turnover of the cytoskeleton may occur at night in the gastroderm of reef-building corals (Mayfield et al., 2010), possibly due to this photosynthetic hysteresis (Levy et al., 2004). Specifically, expression of *actb*, the gene encoding the actin-binding protein tropomyosin, *trp1*, and *tuba* was found to decrease at night in *S. hystrix*, and their expression has been previously shown to also be responsive to temperature changes in other coral species (DeSalvo et al., 2008). Herein, the expression of these cytoskeleton-targeted genes was hypothesized to be similar across temperature treatments of the ETE, yet vary between the stable and variable temperature treatments of the VTE.

Expression of a fourth cytoskeleton gene, *ezrin*, whose respective protein is involved in anchoring the cytoskeleton to the plasma membrane (Rasmussen et al., 2008), and an osmoregulation-targeted gene, *cplap2*, which can catabolize lipids in order to generate the osmolyte glycerol upon decreases in osmotic pressure (Ollivier et al., 2006), was also measured; as with the target genes listed above, they were hypothesized to be expressed at similar levels over time and between treatments in the ETE, yet display temperature treatment differences in the VTE. The final two host coral osmoregulation genes, *trcc* and *oatp*, which encode proteins involved in salt and anion balance, respectively, were also predicted to be stably expressed in the former study and demonstrate temperature treatment differences in the latter. Finally, it was also hypothesized that corals from Houbihu, the UWS, would express higher levels of each of the 14 candidate genes relative to con-specifics from Houwan, the NUWS, for reasons discussed in the Introduction.

3.4 ETE: target gene expression

Twelve genes were targeted in the ETE (Table 4);

Symbiodinium rbcL, *psI* (subunit III), *pgpase*, *nrt2*, and *apx1*, and host coral *actb*, *trp1*, *tuba*, *ezrin*, *cplap2*, *oatp*, and *trcc*. For the latter four genes, primers were designed with CLC Main Workbench (ver. 6.8) from published cDNA sequences (Mayfield et al., 2011). Two additional genes, *Symbiodinium* and host coral *hsp70* were measured in Mayfield et al. (2011), though the data were re-analyzed for the global site of origin comparison described below. The 24 RNAs were diluted to 20 ng μl^{-1}, and 10 µl (200 ng) were converted to cDNA with 1x Solaris RNA spike and the High Capacity™ cDNA synthesis kit (Life Technologies) according to the respective manufacturer's recommendations, precipitated with 0.1 vol sodium acetate (3 M, pH 5.2) and 1 vol isopropanol, and stored at -20℃. At a later date, cDNAs were centrifuged at 12,000 *xg* for 10 min at 4℃, washed once with 75% ethanol, centrifuged at 8,000 *xg* for 5 min, dried on the benchtop after decanting the ethanol, and dissolved in 80 µl diethylpyrocarbonate-treated, double-distilled water. Real-time PCRs (20 µl) were conducted in triplicate on a StepOnePlus™ real-time PCR machine (Life Technologies) with 1x (10 µl) EZ-TIME™ SYBR® Green mastermix with ROX™ passive reference dye (Yeastern Biotech. Co., Ltd.), the primer concentrations found in Table 4, and 2 µl of the 4-fold-diluted cDNA. Serial dilutions of a randomly selected cDNA sample were run on each 96-well plate to estimate the efficiency of each assay (*sensu* Bower et al., 2007), all of which were shown to be between 95-105%. Melt curves were conducted after all reactions in order to verify the absence of non-specific binding.

3.5 VTE: target gene expression

The expression of *Symbiodinium hsp70* and *nrt2*, as well as host coral *actb*, *trp1*, *tuba*, *ezrin*, *cplap2*, *oatp*, *trcc*, and *hsp70* was assessed in the 24 samples of the VTE sampled after seven days. Four additional genes; *rbcL*, *psI* (subunit III), *pgpase*, and *apx1*, were measured in Mayfield et al. (2012a), though the data were re-analyzed for the global site of origin comparison described below. The SYBR Green mastermix, reaction volumes, and real-time PCR instrument described above were used. However, in certain cases (noted in Table 4), slightly different primer concentrations and thermocycling conditions were used between samples of the two experiments.

3.6 Gene expression normalization

For both experiments, the expression of each gene was normalized to Solaris spike recovery as described by Putnam et al. (2013). Then, host and *Symbiodinium* spike-normalized gene expression values were divided by the host and *Symbiodinium* GCPs, respectively; as corals consist of two eukaryotes, both reverse transcription efficiency and biological composition controls are necessary to generate accurate macromolecular expression data (Mayfield et al., 2013b, d). This circumvents the need for housekeeping genes, which cannot be validated with confidence in endosymbiotic organisms (Mayfield et al., 2009). Because DNA samples were pooled and re-purified in the ETE and re-extracted in the VTE, both host and *Symbiodinium* GCPs were recalculated with real-time PCR as described by Mayfield et al. (2012a).

3.7 *Symbiodinium* genotyping

Both previously published (Mayfield et al., 2012a) and unpublished (Ruth D. Gates, personal communication) works have found *S. hystrix* from Southern Taiwan to predominantly possess *Symbiodinium* of clade C. However, as the former study used restriction digests of PCR-amplified *18s* genes and the latter used PCR of the *its2* marker followed by cloning and sequencing, it is possible that background *Symbiodinium* haplotypes may have been overlooked. Real-time PCR, which represents a more sensitive means of detecting cryptic *Symbiodinium* diversity (Mieog et al., 2007), was therefore utilized with all DNAs from both experiments (n=48). Real-time PCRs were conducted for the clade A, C, and D assays of Correa et al. (2009), as these are thought to be the dominant types found in corals of Southern Taiwan (Chen et al., 2005). Reactions (20 µl) were conducted with the mastermix described above for target gene analysis with the primers and primer concentrations of Correa et al. (2009). However, 20 ng of DNA were used in each reaction. Due to the intragenomically variable nature of the *its2* gene (Pochon et al., 2012), these assays cannot be used to calculate exact ratios of the dominant *Symbiodinium* types (e.g., 50% clade A vs. 50% clade C) and are only suited for presence/absence tests. "Presence" was defined *a*

priori as samples in which threshold cycle (C_t) values <30 were calculated.

3.8 VTE: host coral genotyping

It was hypothesized that the dramatic oceanographic differences between Houbihu (UWS) and Houwan (NUWS; Mayfield et al., 2012a), which are only ~15 km apart (Figure 1), could have driven a degree of genetic divergence between coral populations of the two sites of origin. To test this, one of the two pseudo-replicated DNAs from each of the 12 tanks of the VTE (n=6 for each site) was genotyped. Seven species-specific microsatellite loci (Sh4-001, Sh2-002, Sh3-004, Sh2-006, Sh2.15, Sh4.24, Sh4.28; Tables 2 and A4) for *S. hystrix* developed in prior works (Maier et al., 2001, 2005; 2009; Underwood et al., 2006) were utilized to determine the extent of the genetic divergence, if any, between these two populations. Each microsatellite fragment was amplified by PCR (15 μl) in all 12 DNA samples, and reaction mixes contained 10-40 ng DNA, 1x PCR buffer (MDbio), 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 200 nM each primer (Table A4), and 0.1 U *Taq* polymerase (MDbio). Thermocycling was conducted at 95℃ for 3 min followed by 35 cycles of 94℃ for 40 s, 40 s at the annealing temperatures listed in Table A4, and 72℃ for 40 s. A final extension at 72℃ for 1.5 min was then conducted. An Applied Biosystems Genetic Analyzer (3130, Life Technologies) was used to separate fragments, and unique alleles were determined with Peak Scanner™ (ver. 1.0, Life Technologies).

Arlequin (ver. 3.1, Schneider et al., 2000) was used to estimate the N_A, the number of private alleles (N_{PA}), and the H_o and H_e, as well as conduct Hardy-Weinberg equilibrium tests. F_{IS} values were estimated using Fstat ver. 2.9.3.2 (Goudet, 2001). Micro-Checker ver. 2.2.3 (van Oosterhout et al., 2004) was used to detect possible null alleles. Genetic differentiation between populations was estimated by calculating F_{ST} values with Arlequin 3.1. The significance of F_{ST} values for population comparisons was based on 1,000 permutations.

3.9 Statistical analyses

All statistical analyses were conduced with JMP™ (ver. 5, SAS Institute). Shapiro-Wilk W and Levene's tests were used to determine whether datasets were normally distributed and characterized by homogeneous variance, respectively. In certain cases, log, square root, or rank transformations were required to generate data suitable for parametric analyses. To assess differences in both *Symbiodinium* and host gene expression between treatments and over time in the ETE, a repeated-measures ANOVA was conducted with tank nested within treatment after confirming the sphericity of the data with Mauchley's sphericity tests (p>0.05). To assess the effects of site of origin, temperature treatment, and their interaction on both host and *Symbiodinium* gene expression in samples of the VTE, a 2-way ANOVA was conducted with tank nested within site x temperature. In both studies, the tank term was dropped when found to be non-significant (p>0.05), and Tukey's honestly significant difference (HSD) tests were used to verify individual mean differences when significant differences were detected in the model (p<0.05). Correlation analyses were conducted in order to determine the degree of correlation between expression levels of *Symbiodinium* and host coral *hsp70* in samples of the VTE. Slopes were considered significant if p<0.01.

In order to further explore the effect of site of origin on gene expression, the average expression of each gene across the 24 samples from the ETE (all of which were from the UWS) and the 12 samples of the VTE originating from the UWS was compared against the average expression of the 12 samples from NUWS, all of which were from the VTE. This comparison is referred to as the "global site of origin" comparison to distinguish it from the site of origin comparisons within the VTE described above. For the majority of the global site of origin comparisons, Wilcoxon rank-sum tests were utilized, as the variances tended to differ significantly (Levene's test, p<0.01) due to the use of unequal sample sizes (n=36 and 12 for the UWS and NUWS, respectively).

To compare the proportion of the 14 genes in which a significant difference was detected between experiments (ETE vs. VTE) and site of origin (UWS vs. NUWS), X^2 proportion tests were conducted (Quinn and Keough, 2002). Such tests were performed in order to know whether variable temperature

exposure for seven days had a more pronounced effect on gene expression than exposure to a stable, elevated temperature for two days, as well as whether corals of the UWS were more responsive at the mRNA level than those of the NUWS, respectively. Unless otherwise noted, all error terms presented represent standard error of the mean (SEM).

Authors' contributions

ABM conducted the experiments, performed the nucleic acid extractions, executed the real-time PCR assays, and wrote the manuscript. YHC and CFD conducted the host coral genotyping-based laboratory analyses and analyzed the genotyping data. CSC contributed resources and materials that were instrumental to the study's success. All authors read and approved the final manuscript.

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Table A1. ***Symbiodinium*** **exogenous RNA spike recovery, genome copy proportion (GCP), and gene expression results from the** ***Seriatopora hystrix*** **elevated temperature experiment (ETE)**. Repeated-measures ANOVAs were conducted with JMP using a MANOVA model as described in the text. The results from the *S. hystrix* variable temperature experiment (VTE) are presented in the right-most column. "UWS" = upwelling site (Houbihu). "NUWS" = non-upwelling site (Houwan).

Parameter Source of variation	**df**	**Exact *F***	***p***	**Fig.**	**Results from VTE**
Solaris™ spike recovery [a]					
Temperature	1	6.71	0.061	Not shown	
Time	3	7.45	0.121		
Temperature x Time	3	6.19	0.142		interaction effect
Symbiodinium **genome copy proportion (GCP)** [a]					
Temperature	1	0.128	0.739	2A	
Time	3	1.040	0.524		
Temperature x Time	3	0.588	0.679		
ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*) expression [a]					
Temperature	1	0.047	0.839	2B	variable>stable temperature
Time	3	0.528	0.706		
Temperature x Time	3	2.21	0.326		
photosystem I (*psI*; subunit III) expression [a]					
Temperature	1	0.104	0.763	2C	variable>stable temperature
Time	3	0.709	0.630		UWS>NUWS
Temperature x Time	3	0.821	0.590		
phosphoglycolate phosphatase (*pgpase*) expression [a]					
Temperature	1	0.011	0.921	2D	variable>stable temperature
Time	3	0.126	0.937		
Temperature x Time	3	0.276	0.842		
nitrate transporter-2 (*nrt2*) expression					
Temperature	1	0.703	0.449	2E	
Time	3	0.384	0.779		
Temperature x Time	3	0.347	0.800		
ascorbate peroxidase (*apx1*) expression					
Temperature	1	0.053	0.830	2F	
Time	3	0.371	0.786		
Temperature x Time	3	1.81	0.375		

Notes: [a] log-transformed data.

Table A2. **Host genome copy proportion (GCP) and gene expression results from the *Seriatopora hystrix* elevated temperature experiment (ETE).** Repeated-measures ANOVAs were conducted with JMP using a MANOVA model as described in the text. Statistically significant differences are underlined. The results from the *S. hystrix* variable temperature experiment (VTE) are presented in the right-most column.

Parameter Source of variation	**df**	**Exact *F***	***p***	**Fig.**	**Results from VTE**
***S. hystrix* genome copy proportion (GCP)**					
Temperature	1	0.128	0.739	3A	
Time	3	1.040	0.524		
Temperature x Time	3	0.588	0.679		
β-actin (*actb*) expression [a]					
Temperature	1	0.740	0.438	3B	
Time	3	3.64	0.223		
Temperature x Time	3	105.3	<u>0.009</u>		variable>stable temperature
tropomyosin (*trp1*) expression [b]					
Temperature	1	7.33	0.073	3C	
Time	3	3.68	0.362		
Temperature x Time	3	1.093	0.591		
α-tubulin (*tuba*) expression [b]					
Temperature	1	0.492	0.522	3D	
Time	3	1.77	0.381		
Temperature x Time	3	25.0	<u>0.039</u>		
***ezrin* expression** [b]					
Temperature	1	1.24	0.347	3E	
Time	3	2.20	0.452		
Temperature x Time	3	1.76	0.494		
phospholipase-α2 (*cplap2*) expression [b]					
Temperature	1	3.26	0.169	3F	
Time	3	2.23	0.449		
Temperature x Time	3	1.42	0.537		higher in transplanted corals
organic anion transporter (*oatp*) expression [b,c]					
Temperature	1	2.050	0.288	3G	
Time	3	0.264	0.849		
Temperature x Time	3	0.794	0.541		
transient receptor cation channel (*trcc*) expression [b]					
Temperature	1	0.305	0.619	3H	
Time	3	2.040	0.466		
Temperature x Time	3	1.47	0.529		

Notes: [a] rank-transformed data. [b] log-transformed data. [c] univariate tests were used due to lack of data sphericity.

Table A3. ***Symbiodinium*** **and host coral gene expression results from the** ***Seriatopora hystrix*** **variable temperature experiment (VTE).** Two-way ANOVAs were conducted with JMP as described in the text. Statistically significant differences are underlined. "HSD" = honestly significant difference.

Parameter Source of variation	df	MS	*F*	*p*	Tukey's HSD tests	Fig.
Symbiodinium **nitrate transporter-2 (*nrt2*) expression** [a]						
Site of origin	1	0.305	1.65	0.217		4A
Temperature treatment	1	0.753	4.07	0.060		
Site of origin x temperature treatment	1	0.226	1.22	0.285		
Error	17					
Symbiodinium **heat shock protein-70 (*hsp70*) expression** [a]						
Site of origin	1	0.006	0.043	0.839		4B
Temperature treatment	1	1.53	10.70	0.005	variable>stable	
Site of origin x temperature treatment	1	0.081	0.566	0.462		
Error	17	0.143				
S. hystrix **heat shock protein-70 (*hsp70*) expression**						
Site of origin	1	224	0.531	0.476		5A
Temperature treatment	1	17400	41.3	<0.001	stable>variable	
Site of origin x temperature treatment	1	1230	2.91	0.107		
Error	17	422				
S. hystrix **β-actin (*actb*) expression** [a]						
Site of origin	1	0.018	0.340	0.567		5C
Temperature treatment	1	0.349	6.68	0.019	variable>stable	
Site of origin x temperature treatment	1	0.137	2.62	0.124		
Error	17	0.052				
S. hystrix **tropomyosin (*trp1*) expression**						
Site of origin	1	124	1.79	0.199		5D
Temperature treatment	1	97.8	1.41	0.252		
Site of origin x temperature treatment	1	34.1	0.491	0.493		
Error	17	69.5				
S. hystrix **α-tubulin (*tuba*) expression**						
Site of origin	1	83.7	0.021	0.887		5E
Temperature treatment	1	6200	1.55	0.230		
Site of origin x temperature treatment	1	9860	2.47	0.135		
Error	17	3400				
S. hystrix ezrin expression						
Site of origin	1	2540	2.36	0.143		5F
Temperature treatment	1	1.34	0.001	0.972		
Site of origin x temperature treatment	1	382	0.356	0.559		
Error	17	1072				
S. hystrix **phospholipase-α2 (*cplap2*) expression**						
Site of origin	1	1.92	4.14	0.058		5G
Temperature treatment	1	0.004	0.009	0.926		
Site of origin x temperature treatment	1	2.21	4.75	0.044	NUWS-variable>UWS-variable	
Error	17	0.464				
S. hystrix **organic anion transporter (*oatp*) expression**						
Site of origin	1	0.064	0.016	0.902		5H
Temperature treatment	1	2.90	0.712	0.411		
Site of origin x temperature treatment	1	12.1	2.97	0.103		
Error	17	4.069				
S. hystrix **transient receptor cation channel (*trcc*) expression** [a]						
Site of origin	1	0.001	0.004	0.948		5I
Temperature treatment	1	0.074	0.515	0.483		
Site of origin x temperature treatment	1	0.113	0.787	0.387		
Error	17	0.144				

Notes: [a] log-transformed data.

Table A4. Microsatellite analysis of host corals from the variable temperature experiment (VTE). Seven microsatellite loci were assessed in order to genotype the 12 *Seriatopora hystrix* colonies (6 from the upwelling site [UWS; i.e., Houbihu] and 6 from the non-upwelling site [NUWS; i.e., Houwan]) of the VTE. Additional details about the microsatellites (e.g., fluorescent labeling of the respective primers) can be found in the manuscripts in which they were first published (Maier et al., 2001; 2005; 2009; Underwood et al., 2006). "F" and "R" are abbreviations for forward and reverse, respectively.

Locus	NCBI accession number	Primer sequences ('5-3')	Motif	Annealing temperature (℃)
Sh4-001	DQ131572	F: ACTTCATTCAATTATCCATAAAGTCAT R: ATCACTGATTAGACTTACAAACAAACTG	$(CATT)_{10}$	61
Sh2.15	AY604005	F: CGTGCCACTGTGATTTCTTC R: AACAAAAACGTCTCCATTACCC	$(CA)_{18}$	60
Sh2-002	DQ131573	F: GTGAATAAGAACGACGGA R: AAATACTAATTACAGGCATGAC	$(CA)_{18}$	60
Sh4.28	AF320769	F: TGTGGTCTACAGTATATCTTTTGTG R: CAAATTTGAATCTACAGTGGGG	$(CA)_{11}$	61
Sh2-006	DQ131577	F: CAAATACCAATCAGTGTAGCA R: GGCCTAATATCTGTCTCCTTC	$(CT)_8$, TT, $(CT)_7$	61
Sh4.24	AY604006	F: TCCTCCAGATGAATTTGAACG R: TTCAGGGAAGATTTGCCG	$(CA)_{37}$	61
Sh3-004	DQ131575	F: GGTTACTTATGTGTTTTGCTT R: AGTTGATGTTGTTGCCTT	$(AAC)_{12}$	53