Expression of *hsp70*, *hsp90* and *hsf1* in the reef coral *Acropora digitifera* under prospective acidified conditions over the next several decades

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Summary

Ocean acidification is an ongoing threat for marine organisms due to the increasing atmospheric CO_2 concentration. Seawater acidification has a serious impact on physiologic processes in marine organisms at all life stages. On the other hand, potential tolerance to external pH changes has been reported in coral larvae. Information about the possible mechanisms underlying such tolerance responses, however, is scarce. In the present study, we examined the effects of acidified seawater on the larvae of *Acropora digitifera* at the molecular level. We targeted two heat shock proteins, Hsp70 and Hsp90, and a heat shock transcription factor, Hsf1, because of their importance in stress responses and in early life developmental stages. Coral larvae were maintained under the ambient and elevated CO_2 conditions that are expected to occur within next 100 years, and then we evaluated the expression of *hsp*s and *hsf1* by quantitative real-time polymerase chain reaction (PCR). Expression levels of these molecules significantly differed among target genes, but they did not change significantly between CO_2 conditions. These findings indicate that the expression of *hsp*s is not changed due to external pH changes, and suggest that tolerance to acidified seawater in coral larvae may not be related to *hsp* expression.

Key words: larvae, scleractinian corals, heat shock proteins, ocean acidification

Introduction

Ocean acidification is a result of anthropogenic climate change and is challenging marine ecosystems (Hoegh-Guldberg and Bruno, 2010). The surface layers of the ocean are acidifying due to the absorption of anthropogenic CO_2 and have already absorbed approximately one-third of the human-produced CO_2, resulting in a decrease in the ocean pH by 0.1 pH unit from the pre-industrial era (Doney et al., 2009). Atmospheric CO_2 concentration, which is currently 380 ppm, is expected to rise to 1000 ppm by the end of the 21st century (Raven et al., 2005). Therefore, the ocean pH is expected to further decrease 0.3 to 0.5 pH units by 2100 (Caldeira and Wickett, 2005).

Seawater acidification has a serious impact on the physiologic processes in marine organisms throughout life, including fertility (e.g. Kurihara and Shirayama, 2004; Havenhand et al., 2008; Albright et al., 2010), calcification and/or growth rate (e.g. Fine and Tchernov, 2007; Kurihara et al., 2007; Dupont et al., 2008), metabolism (e.g. Reipschläger and Pörtner, 1996; Michaelidis et al., 2005), and metamorphosis/settlement (e.g. Albright et al., 2010; Nakamura et al., 2011). On the other hand, the survival of coral larvae are not obviously impacted by acidified seawater (Suwa et al., 2010; Nakamura et al., 2011), suggesting that coral larvae have some level of tolerance to external pH changes. The potential for metabolism suppression, a suggested survival strategy under stressful conditions (Guppy and Withers, 1999), has been reported for coral larvae (Nakamura et al., 2011). Information on the mechanisms underlying a tolerance response to acidified seawater, however, is scarce. Only a few studies have focused on the responses to environmental pH changes at the molecular level in marine invertebrates (Hauton et al., 2009; O'Donnell et al., 2009; Todgham and Hofmann, 2009; O'Donnell et al., 2010) and none of these studies have focused on corals, although profiling and quantifying gene expression will strongly enhance our understanding of the physiologic response of marine organisms in an acidifying ocean.

Heat shock proteins (Hsps) are involved in cellular defense and morphologic changes of organisms at developmental stages (Feder and Hofmann, 1999; Gunter and Degnan, 2007). Although not all Hsps are stress-inducible, they respond to various environmental stressors such as extreme temperatures, pH extremes, anoxia, and various toxic substances (Feder and Hofmann, 1999; Dahlhoff, 2004). Such *hsp*s expression is mainly regulated by heat shock factors (HSFs) (Sorger, 1991; Pirkkala et al., 2001).

Here, we observed the transcription levels of two Hsps (*hsp70* and *hsp90*) and an HSF (*hsf1*) when larvae of the scleractinian coral *Acropora digitifera* were exposed to acidified seawater for 1 to 7 days after fertilization. We also evaluated the expression

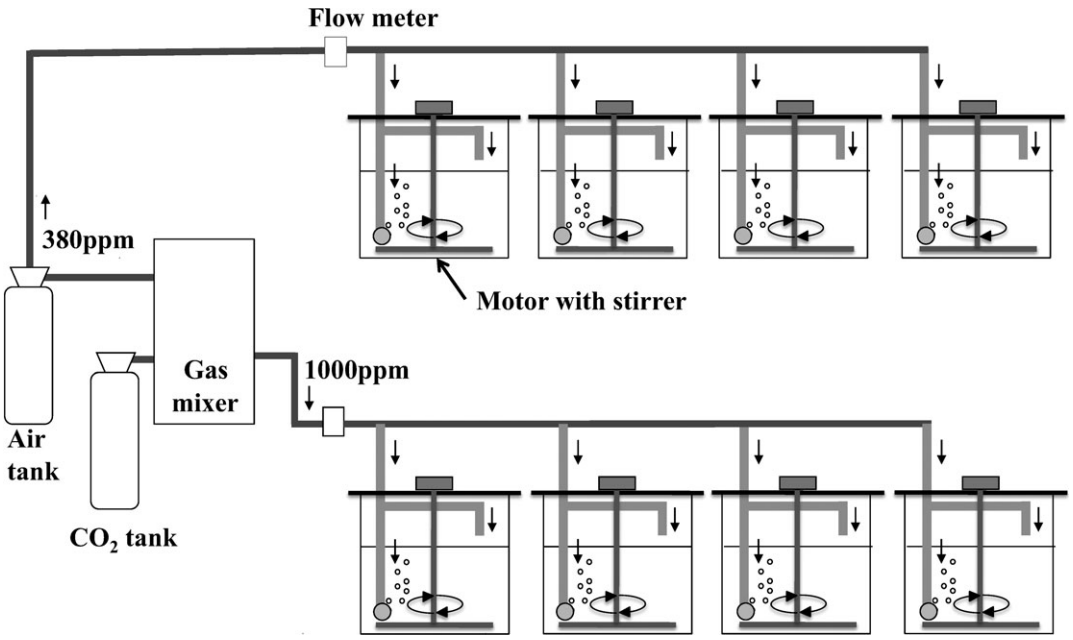


Fig. 1. Schematic of the aquarium settings for the pH treatment.

pattern of *hsp*s during early developmental stages in the coral *A. digitifera* under normal conditions, the first study of its kind to be performed in corals.

Among the Hsp and HSF groups, we focused on the expression of *hsp70*, *hsp90,* and *hsf1* under acidified conditions to gain insight into the response to ocean acidification in coral larvae at the molecular level due to the importance of these genes in the stress response and in early life development. Hsp70 and Hsp90 are generally considered to contribute to stress responses (Freeman and Morimoto, 1996; Feder and Hofmann, 1999). In addition, their expression seems to be related to the activation of HSF1 (Morimoto, 1998; Zou et al., 1998). Hsp90s also have an important role in regulating metamorphosis and developmental plasticity in relation with environmental conditions (Bishop et al., 1998; Rutherford and Lindquist, 1998; Bishop et al., 2001; Queitsch et al., 2002; Baker, 2006). Therefore, evaluating the expression of *hsp70*, *hsp90,* and *hsf1* is a good starting point for investigating the relevancy of *hsp*s expression in corals under acidified conditions with quantitative analyses.

Materials and Methods

Larval culture

A scleractinian coral species, *Acropora digitifera,* was used in the present study. This coral species distributes commonly around Okinawa coral reefs and is also the most commonly used species in studies of the effects of acidified seawater on several early life stages in coral (Morita et al., 2010; Suwa et al., 2010; Nakamura et al., 2011). This species is also an important model species because their genome has recently been sequenced (Shinzato et al., 2011).

Colonies of *A. digitifera*, identified as being sexually matured, were collected from fringing reefs around Sesoko Island, Okinawa, Japan (26°38′N, 127°53′E) one week before their predicted spawning date in June 2010. They were maintained in a running seawater tank under natural light conditions at Sesoko Station, Tropical Biosphere Research Center, University of the Ryukyus.

Gametes were collected from five colonies on spawning nights according to Morita et al. (Morita et al., 2006), mixed, and fertilized. The fertilized gametes were kept in a container filled with filtered seawater (FSW, pore size 1 μm).The FSW was exchanged at least twice per day before the experiment started to keep the gametes and/or larvae healthy.

Four aquariums (5L) with a cover were prepared for control and high pCO_2 (1000 ppm) conditions (Fig. 1). The CO_2 concentration of the seawater in the aquariums was controlled by aerating the FSW with air (control) or high CO_2 concentration gas (1000 ppm). The high CO_2 gas was produced by controlling the flow rate of air and pure CO_2 using mass flow controllers (Horiba STEC, Air: SEC-E50, CO2: SEC-E40). The seawater and air layer of the aquariums were continuously aerated by the air or high CO_2 gas to maintain the partial pressure of CO_2 concentrations (P_{CO2}) in the seawater at equilibrium. A flow meter (Kofloc RK-1250, Kyoto, Japan) was used to adjust the flow rate of air and high CO_2 gas for each aquarium. Temperature of the seawater in each aquarium was maintained at 27℃ with water-coolers (REI-SEA LX-120EXA, IWAKI CO., Tokyo, Japan) and electric water-heaters.

Approximately 1500 larvae at 1 day post-fertilization (1 dpf) were placed in each aquarium with 3.2 L control or high CO_2 FSW. The seawater of each aquarium was exchanged once per day with pre-treated seawater aerated with air (control) or high CO_2 concentration gas (1000 ppm). At 1, 3, 5, and 7 days after culture, approximately 200 larvae were sampled from each aquarium for the experiments. Larvae of *A. digitifera* are lecithotrophic and they do not contain algal symbionts (zooxanthellae).

The pH (NBS scale) and temperature of the seawater were measured every day using a pH meter (Mettler Toledo, Seven Go). The salinity and total alkalinity were measured every 2 days using a salinometer (AS ONE, IS/Mill-E) and a total alkalinity titration analyzer (Kimoto ATT-05, Japan), and seawater carbonate chemistry was calculated using the program CO2SYS_calc_XLS (Pierrot et al., 2006) (Table 1).

RNA extraction

Total RNA was extracted from a sample of approximately 200 larvae per tube. There were four replicates for each pH treatment for each observational day. Larvae were first fixed with RNA stabilization solution, RNAlater (Ambion), and

Table 1. Summary of physical conditions in the experimental aquaria at different CO_2 conditions.

CO_2 condition	pH	Temperature (℃)	Salinity (‰)	TA (μmol/kg)	pCO_2[a] (μatm)	$HCO3^-$ (μmol/kg)	CO_3^{2-} (μmol/kg)	Ω_{Arag}
Control (380 ppm)	8.13 ± 0.01	27.0 ± 0.0	35.0 ± 0.0	2255.1 ± 3.9	492.3 ± 24.0	1776.9 ± 16.6	194.1 ± 6.2	3.0 ± 0.1
1000 ppm	7.89 ± 0.00	27.0 ± 0.0	35.0 ± 0.0	2255.9 ± 4.2	925.6 ± 10.8	1954.9 ± 3.9	122.1 ± 0.9	1.9 ± 0.0

stored at –80℃. Total RNA was then isolated using TRIzol Reagent (Invitrogen), following treatment with chloroform and 2-propanol. Total RNA pellets were then washed with 75% ethanol and dried. Dried total RNA pellets were resuspended in 25 µl nuclease-free water. RNA was quantified spectrophotometrically using an ND-1000 UV/visible spectrophotometer (NanoDrop technologies). TURBO DNA-free™ Kit (Applied Biosystems) was then used to remove contaminating DNA from the RNA preparations. cDNA was synthesized with AMV reverse transcriptase from total RNA prepared as described above, using a TaKaRa RNA PCR kit (AMV) Ver.3.0.

Quantitative real-time PCR analysis

Real-time PCR reactions were conducted in a total volume of 20 µl comprising 10 µl of Mastermix with SYBR green (Bio-Rad), 0.6 µl of each primer (1.2 µM), 0.3 µL of sample cDNA, and 8.5 µl ultrapure water. Real-time PCR reactions were run on a Thermal Cycler Dice Real time system TP800 (TAKARA BIO INC., Shiga, Japan) under the following reaction conditions: 1 cycle of [95℃ for 3 min], 45 cycles of [94℃ for 30 s → 55℃ for 30 s → 72℃ 30 s], and 1 cycle of [95℃ for 15 s → 60℃ for 30 s → 95℃ for 15 s]. Data were normalized against the expression of actin, which was confirmed not to vary in response to CO_2 over the observational days by two-way factorial analysis of variance (ANOVA).

Primers were designed with Primer 3 (v. 0.4.0) software based on the partial sequence of each target gene of *A. digitifera* (Table 2).

Relative concentrations of mRNA for each pH treatment per observation day (x) were calculated using the following equation:

$$x = \left[\sum_{i}^{m} 1/2^{(\mathrm{exCt\text{-}refCt})_i}\right] \Big/ 4$$

where i=aquarium number (1 to 4), m=4 (there are four aquariums for each pH treatment), exCt=experimental Ct, and actCt=Ct of reference gene (actin).

Rapid amplification of 5′ and 3′ cDNA ends (RACE) and phylogenetic-tree construction

The SMARTer™ RACE cDNA Amplification Kit (Clontech) was used for generating 5′ and 3′ cDNA sequences of *hsp70*, *hsp90,* and *hsf1*. Gene-specific 5′ and 3′ RACE and nested RACE primers for each gene were designed based on the nucleotide sequences of each gene fragment. The generated RACE-ready 5′ and 3′ cDNA, the kit reagents, and total RNA were used. First, PCR reactions were performed for each cDNA end for each gene using the universal primer (UPM) and the gene-specific RACE primers. Then, nested RACE reactions were conducted for each end of each gene by diluting the 1st PCR products as a template with the nested universal primer (NUP) and nested gene-specific primers. Conditions for both the 1st RACE and nested RACE PCR reactions were as follows; 5 cycles of [94℃ for 30 s → 72℃ for 3 min], 5 cycles of [94℃ for 30 s → 74℃ for 30 s → 72℃ for 3 min], and 35 cycles of [94℃ for 30 s → 68℃ for 30 s → 72℃ for 3 min] with a final extension of 72℃ for 3 min. The products of the 5′ and 3′ nested RACE PCR were gel-purified with Wizard SV Gel and PCR Clean-Up System (Promega), cloned into pGEM-T easy (Promega), and sequenced. The resulting sequences were compared to GenBank using a BLAST program: the nucleotide blast. The same processes were performed until the full sequence of each gene was obtained. Maximum-likelihood phylogenetic analyses were performed with PAUP*4.0b10 (Swofford, 2003). The appropriate model parameters for the maximum-likelihood analysis were determined using a likelihood-ratio test with Modeltest version 3.7 (Posada and Crandall, 1998; Posada and Buckley, 2004). The tree was searched using neighbor-joining methods based on maximum-likelihood distances from a best-fit model of nucleotide substitutions as determined by Modeltest.

Statistical analysis

Expression levels of the genes were compared among *hsp70*, *hsp90,* and *hsf1* over observation days under the control CO_2 condition using a two-way factorial ANOVA. To examine the effects of elevated CO_2 on the expression of *hsp70*, *hsp90,* and *hsf1*, we performed a two-way factorial ANOVA with CO_2 treatment and observation day as factors. Tukey's HSD multiple comparison tests were conducted as a *post-hoc* test when the ANOVA detected significant differences. Data were log-transformed to meet normality and homoscedacity of variance criteria where necessary. All statistical analyses were performed by software JMP version 8.0.2.

Table 2. Primers for quantitative real-time PCR.

Gene	Sense	Antisense
hsp70	ATCAATTCCCTC-AGCCAGTG	AGCGGATCTAAAG-GGAAACC
hsp90	CTTTTCGGTTGAA-GGTCAGC	GGAATGATGTCC-TCGCAGTT
hsf1	CCAGCTTCCACAG-TTTCACA	TTCCTACAACAC-ACGCGAAC
Actin1	GCAAAAGGAAATT-GCTGCTC	CACATCTGTTGGAA-GGTGGA

Results

The genes coding for a 70-kDa heat shock protein (*hsp70*), a 90-kDa heat shock protein (*hsp90*), and a heat shock transcription factor1 (*hsf1*) were identified in the scleractinian coral, *Acropora digitifera*. The *hsp70* was 1633 nucleotides long, encoding a 219-amino acid protein in the open reading frame (ORF). A phylogenetic analysis demonstrated that the *hsp70* of a target species, *A. digitifera,* was structurally similar to other scleractinian corals (Fig. 2a). The *hsp90* was 2554 nucleotides long, encoding a 733-amino acid protein in the ORF. According to a phylogenetic analysis, the structure of *hsp90* was similar to that in other cnidarians (Fig. 2b). The *hsf1* was 2507 nucleotides long with an encoded 504-amino acid protein in the ORF. The structure of *hsf1* was also structurally similar to that of other cnidarians (Fig. 2c).

Comparison of the expression of three target genes under the control CO_2 condition

Transcripts of the three target genes were detected from Day 1 (2 dpf). The expression of these three target genes under the control condition differed significantly among the three genes through the experiment (Table 3) (Fig. 3). Expression of *hsp90* was significantly higher than that of the two other genes throughout the experiment. Expression of *hsp90* on Day 1 was more than 50-fold higher than that of the other genes and nearly 5-fold more than that of the other observation days for *hsp90* (Tukey HSD test, $P<0.05$). Expression of *hsp70* was relatively low throughout the experiment. The expression of *hsf1* was bit higher on Day 5 (6 dpf) than on the other days.

Expression of hsp70 under elevated CO_2

Expression of *hsp70* was not significantly different between CO_2 conditions throughout the period of observation (ANOVA, $F_{CO_2\ conditions \times Day}=1.1438$, $p=0.351$, Fig. 4). Expression under the elevated CO_2 condition during the first 2 days of observation (Day1 [2 dpf] and Day3 [4 dpf]) was relatively higher than that under the control condition and the other days under the elevated CO_2 condition.

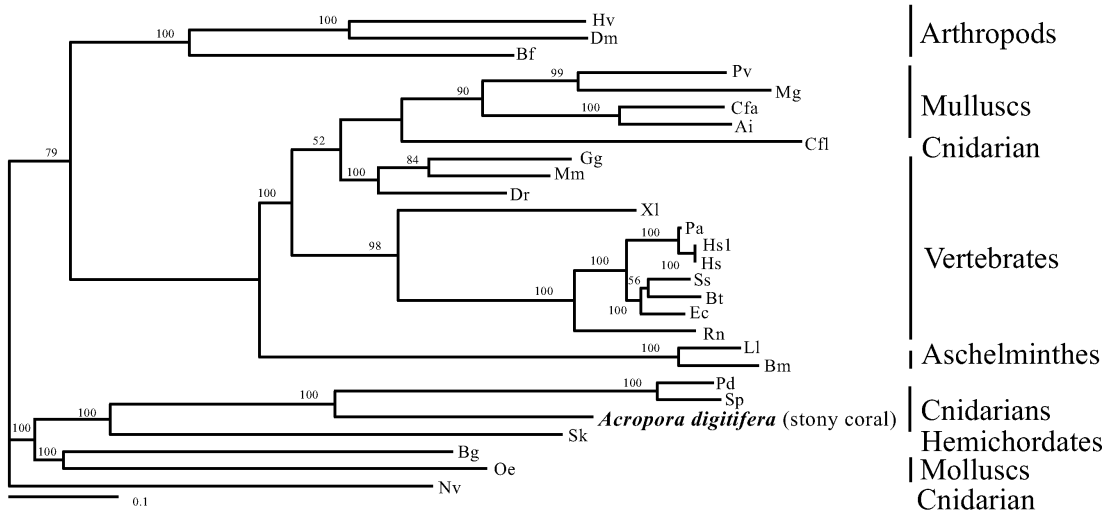
Expression of hsp90 under elevated CO_2

Expression of *hsp90* did not differ considerably between CO_2 conditions throughout the experiment, whereas expression was somewhat higher in the control condition than in the elevated CO_2 condition (Table 4) (Fig. 5). Expression was significantly higher on Day 1 than on the other days (Tukey HSD test, $P<0.05$), and then decreased on Day 3, and maintained a low level over the following days.

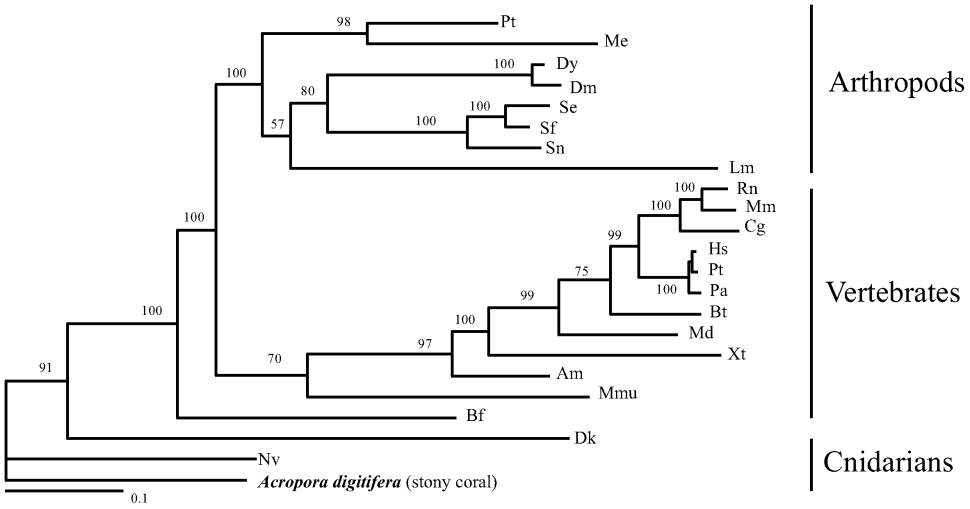
Expression of hsf1 under elevated CO_2

Expression of *hsf1* did not vary significantly between CO_2 conditions throughout the observation (ANOVA, $F_{CO_2\ conditions \times Day}=0.8503$, $p=0.48$, Fig. 6). The expression patterns were not consistent between CO_2 conditions. In the elevated CO_2 condition, expression showed a downward trend over time, whereas expression under the control condition was erratic,

a) Hsp70



b) hsp90



c) hsf1

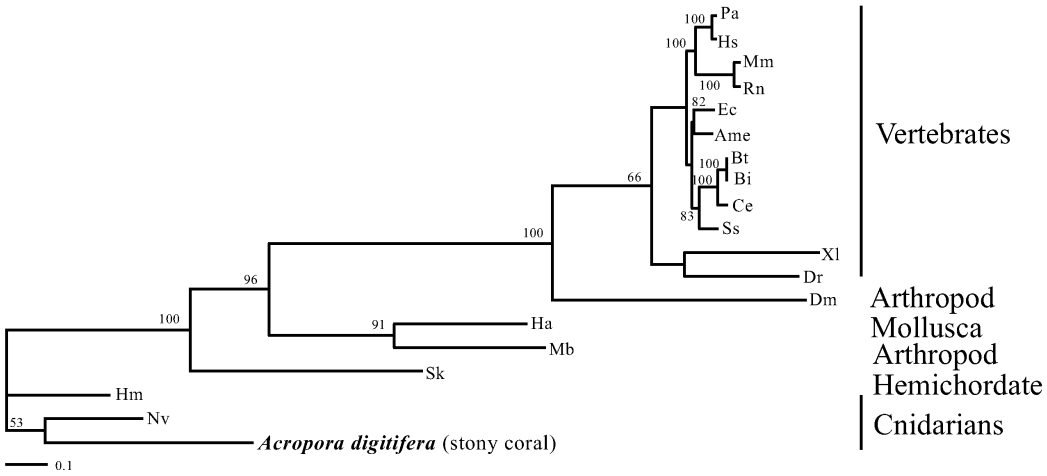


Fig. 2. See next page for legend.

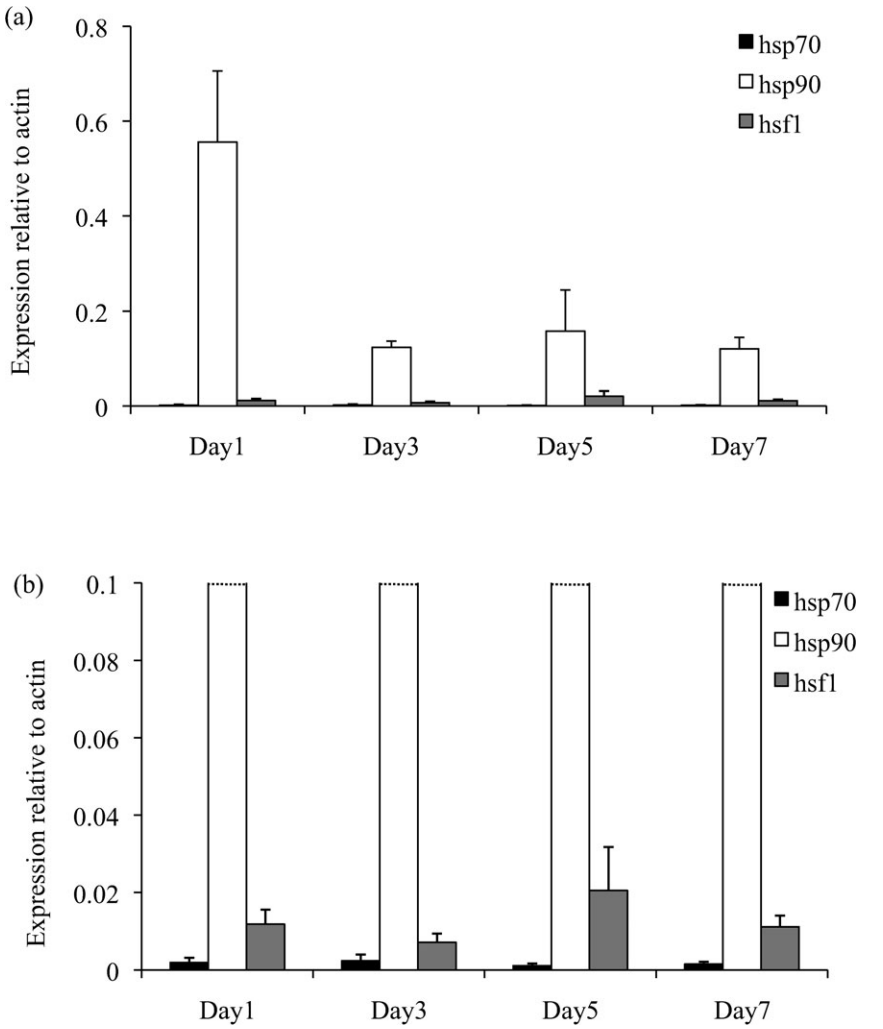


Fig. 3. Expression of *hsp70*, *hsp90*, and *hsf1* in the larvae of cultured *Acropora digitifera* in the control CO_2 condition (380 ppm) at 27℃ over 7 days. (A) Overall view of the expression, (B) Enlarged view of y-axis from 0 to 0.1 of Fig. 3A. Error bars indicate mean and standard error (N=4).

Table 3. Two-way factorial ANOVA on the gene expression of larvae of *Acropora digitifera* in the control CO_2 condition (380 ppm).

Source	*Df*	Sum of squares	*F*	*p*
Gene	2	0.57723	28.1457	< 0.0001
Day	3	0.17905	5.8206	0.0024
Gene × Day	6	0.35831	5.8239	0.0003

showing a tendency to decrease during the first two days, then an increase at Day 5, and a decrease at Day 7.

Discussion

In the present study, we evaluated the expression of *hsp70*, *hsp90*, and *hsf1* to determine whether theses genes are involved in the tolerance response against acidified conditions during the

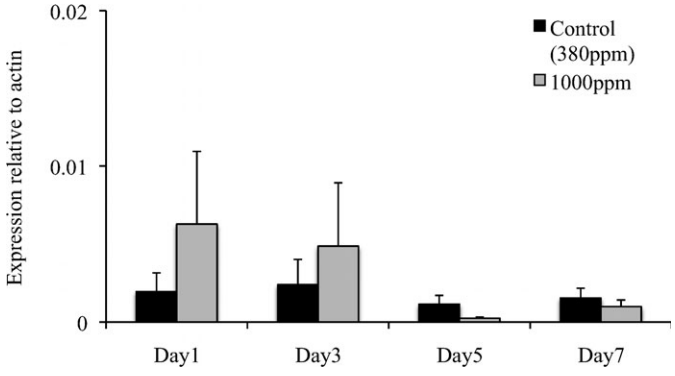


Fig. 4. Expression of *hsp70* in the larvae of cultured *Acropora digitifera* in the control and elevated CO_2 conditions at 27℃ over 7 days. Error bars indicate mean and standard error (N=4).

Fig. 2. Phylogenetic trees of hsp70, hsp90, and hsf1 amino acid sequences. (A) hsp70, (B) hsp90, and (C) hsf1. [Hv] Heliothis viriplaca, [Dm] Drosophila melanogaster, [Bf] Branchiostoma floridae, [Pv] Perna viridis, [Mg] Mytilus galloprovincialis, [Cfa] Chlamys farreri, [Ai] Argopecten irradians, [Cfl] Chironex fleckeri, [Gg] Gallus gallus, [Mm] Mus musculus, [Dr] Danio rerio, [Xl] Xenopus laevis, [Pa] Pongo abelii, [Hs1] Homo sapiens heat shock 70 kDa protein 1-like, [Hs] Homo sapiens, [Ss] Sus scrofa, [Bt] Bos taurus, [Ec] Equus caballus, [Rn] Rattus norvegicus, [Ll] Loa loa, [Bm] Brugia malayi, [Pd] Pocillopora damicornis, [Sp] Stylophora pistillata, [Sk] Saccoglossus kowalevskii, [Bg] Biomphalaria glabrata, [Oe] Ostrea edulis, [Nv] Nematostella vectensis, [Pt] Portunus trituberculatus, [Me] Metapenaeus ensis, [Dy] Drosophila yakuba, [Se] Spodoptera exigua, [Sf] Spodoptera frugiperda, [Sn] Sesamia nonagrioides, [Lm] Locusta migratoria, [Cg] Cricetulus griseus, [Pt] Pan troglodytes, [Md] Monodelphis domestica, [Xt] Xenopus tropicalis, [Am] Alligator mississippiensis, [Mmu] Mscaca mulatta, [Dk] Dendronephthya klunzingeri, [Ame] Ailuropoda melanoleuca, [Bi] Boa indicus, [Ce] Cervus eldi, [Ss] Sus scrofa, [Dr] Danio rerio, [Ha] Haliotis asinine, [Mb] Mamestra brassicae, [Hm] Hydra magnipapillata.

Table 4. Two-way factorial ANOVA on the *hsp90* expression of larvae of *Acropora digitifera* in the different CO_2 conditions.

Source	*Df*	Sum of squares	*F*	*p*
CO_2 condition	1	0.05319	0.5169	0.479
Day	3	3.26178	10.5663	< 0.001
CO_2 condition × Day	3	0.07171	0.2323	0.872

larval stage in corals. Larvae are considered to have a number of cellular defense mechanisms to buffer the effects of environmental stressors (Goldstone et al., 2006; Hamdoun and Epel, 2007). If the expression of *hsp*s and *hsf* change in acidified conditions, these stress proteins are likely to be involved in the stress response to acidified conditions (Feder and Hofmann, 1999; Dahlhoff, 2004).

In contrast to our hypothesis, the expression of *hsp*s in coral larvae may not be affected by an external pH change. Our findings demonstrated that the expression levels of *hsp70*, *hsp90*, and *hsf1* in larvae of *Acropora digitifera* were poorly differentiated between the control and acidified conditions (Figs 4, 5, and 6). These findings indicate that the pH treatments do not induce heat shock proteins to refold some denatured cytoplasmic proteins by an acid/base imbalance due to external pH changes. Similar to our results, Hauton et al. (Hauton et al., 2009) reported no significant effect of acidified seawater on the expression of *hsp70* in the neritic amphipod *Gammarus locusta*, suggesting that physiologic buffering mechanisms are present in the tissues of *G. locusta* to cope with a decrease in external pH. Larvae of *A. digitifera* should be able to cope with the pH decrease that is expected to occur in this century at the cellular level.

Our next step will be to investigate effects of multiple environmental factors simultaneously to clarify the effects of acidified seawater on *hsp* expression in coral larvae, which was beyond the scope of the present study. For example, expression of *hsp70* in sea urchin larvae with a simultaneous temperature increase differs among pCO_2 conditions (380 ppm–970 ppm) (O'Donnell et al., 2009). Therefore, in sea urchin larvae, the stress response to thermal stressors is altered according to the acidified conditions.

Although the expression of *hsp*s and *hsf1* did not change significantly under the acidified conditions, their expression was significantly different among genes under control conditions.

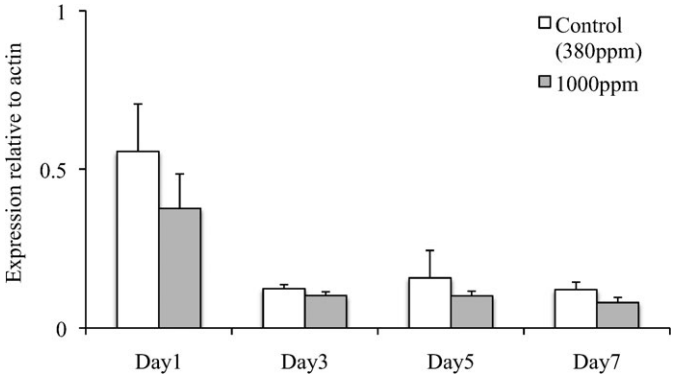


Fig. 5. Expression of *hsp90* in the larvae of cultured *Acropora digitifera* in the control and elevated CO_2 conditions at 27℃ over 7 days. Error bars indicate mean and standard error (N=4).

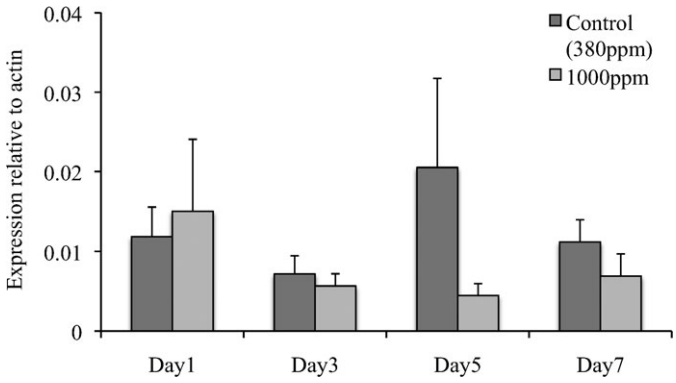


Fig. 6. Expression of *hsf1* in the larvae of cultured *Acropora digitifera* in the control and elevated CO_2 conditions at 27℃ over 7 days. Error bars indicate mean and standard error (N=4).

Hsp70, Hsp90, and HSF1 function differently at early life stages, including the larval stage. For example, Hsp70 is toxic in early development under ambient or non-stressful conditions (Krebs and Feder, 1997), as opposed to its beneficial effects on survival under stressful conditions (Feder et al., 1992). A low abundance of *hsp70* at larval stages was previously reported for the fruit fly and the silver sea bream (Velazquez et al., 1983; Deane and Woo, 2003), such as the coral *A. digitifera* (Fig. 3). This may explain the comparatively low expression of *hsp70* in the present study.

Contrary to the toxic characteristics of Hsp70 in early life stages, Hsp90 is among the most abundant proteins in the cytosol of eukaryotic cells (Mayer and Bukau, 1999), and the expression of *hsp90* is observed from very early developmental stages under normal unstressed conditions (e.g. Ali et al., 1998; Deane and Woo, 2003; Manchado et al., 2008). Hsp90 is also considered to control cell growth and differentiation (e.g. Krone et al., 1997; Mayer and Bukau, 1999; Deane and Woo, 2003; Krone et al., 2003) and to be involved in hormonal control, which regulates developmental changes in early development of larvae (e.g. Deane and Woo, 2003). Correspondingly, relatively higher expression levels of *hsp90* were detected in the present study, suggesting the importance of Hsp90 during early developmental stages.

In response to a multitude of stress conditions, HSF1 acquires DNA binding activity to the heat shock element, leading to the accumulation of Hsps (Wu, 1995; Morimoto, 1998). For instance, the accumulation of Hsp70 leads directly to the binding of Hsp70 to the HSF1 activation domain, and results in the repression of heat shock-induced transcription. This reaction subsequently converts HSF1 trimers to monomers, losing HSF1 DNA binding and then reading, to attenuate the heat shock response (Shi et al., 1998). Thus, Hsp70 may have a role in regulating HSF1 deactivation (Abravaya et al., 1992; Shi et al., 1998). As another example, Hsp90 has a role in maintaining HSF1 in an inert state (Ali et al., 1998; Zou et al., 1998; Bharadwaj et al., 1999). Therefore, these processes may account for the expression profiles of the three target genes in the present study; a relatively high expression of *hsp90* and substantially low expression of *hsp70* and *hsf1* throughout the experiment.

In conclusion, our findings are the first to demonstrate the expression patterns of *hsp*s and *hsf1* during larval development in coral by observing the transcription levels of two Hsps and an HSF of coral larvae under acidified conditions. These findings

suggest that coral larvae are able to buffer the external pH changes that are expected to occur by the end of this century.

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