ORIGINAL ARTICLE

Temperature relations of aerial and aquatic physiological performance in a mid-intertidal limpet Cellana toreuma: Adaptation to rapid changes in thermal stress during emersion

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Abstract

The physiological performance of a mid-intertidal limpet Cellana toreuma was determined to study the physiological adaptation of intertidal animals to rapid changes and extreme temperatures during emersion. The relationship between the Arrhenius breakpoint temperature (ABT) and in situ operative body temperature was studied to predict the possible impact of climate change on the species. The temperature coefficient (Q10) of emersed animals was higher than that of submersed animals and the ratio of aerial: aquatic heart rate rose with increasing temperature. The ABTs of submersed and emersed animals were 30.2 and 34.2°C, respectively. The heart rate and levels of molecular biomarkers (hsp70, ampka, ampkβ and sirt1 mRNA) were determined in 48 h simulated semi-diurnal tides. There were no obvious changes of heart rate and gene expression during the transition between emersion and submersion at room temperature, although expressions of hsp70 and hsp90 were induced significantly after thermal stress. These results indicate that C. toreuma can effectively utilize atmospheric oxygen, and the higher Q10 and ABT of emersed animals are adaptations to the rapid change and extreme thermal stress during emersion. However, the in situ operative body temperature frequently exceeds the aerial ABT of C. toreuma, indicating the occurrence of large-scale mortality of C. toreuma in summer, and this species should be sensitive to increasing temperature in the scenario of climate change.

Key words: climate change, intertidal, limpet, physiological adaptation, temperature

INTRODUCTION

Intertidal animals have to deal with environmental conditions that range from fully submersed to fully terrestrial over vertical distances of a few meters (McMahon 1988; Helmuth et al. 2002; Harley & Helmuth 2003; Little et al. 2009). Because most intertidal molluscs are sessile or have limited mobility, they have to suffer the rapid changes of various environmental fac-
tors including extreme temperatures (McMahon 1988). Physiological adaptations to the highly variable abiotic factors play crucial roles in determining their intertidal zonation (Wolcott 1973; Branch 1981; Garrity 1984; McMahon 1990; Little et al. 2009) and in determining their future population dynamics in the scenario of climate change (Somero 2002; Pörtner & Knust 2007; Somero 2012). The aerial and aquatic thermal regimes for intertidal animals are dramatically different due to the tidal cycle. The change of temperature underwater is relatively benign; however, the change of temperature in air is rapid and extreme. Therefore, intertidal animals have to develop effective strategies to survive the different thermal regimes (Denny et al. 2011).

The ability of intertidal molluscs to utilize atmospheric oxygen has been studied for a long time (e.g. Newell 1973; Bannister 1974; McMahon 1988; McMahon et al. 1991; Marshall & McQuaid 1992a). The physiological responses to extreme temperatures are different for intertidal molluscs inhabiting different tidal regions (Newell 1979; Little et al. 2009). Usually, the ratios of aerial and aquatic O₂ consumption rates for high-intertidal molluscs are higher than those in mid-intertidal and low-intertidal animals, allowing them to cope with the longer air exposure in the high intertidal zone (McMahon & Russell-Hunter 1977; McMahon 1988). However, the trade-offs that intertidal molluscs make between aerobic and anaerobic metabolism during emersion and their relationship to desiccation stress are species-specific and not always clear (Marshall & McQuaid 1992a; Somero 2002).

Heart rate of intertidal molluscs is affected by various factors (Santini et al. 2000) and can be used to infer metabolic rate in gastropods (Santini et al. 1999). The aerial heart rate and oxygen consumption were linearly correlated for limpets Patella granularis and Siphonaria oculus (Marshall & McQuaid 1992b). The heart rates of emersed inactive animals were positively related to air temperature; however, there was no positive relationship between the heart rate of submersed limpets and water temperature (Santini et al. 2000). Extensive studies showed that the cardiac performances of intertidal gastropods are closely related to the animals' vertical zonation (Chelazzi et al. 2001; Stenseng et al. 2005; Dong & Williams 2011).

The fine balance between stability and lability that correlated with adaptation temperatures in proteins is a strikingly consistent feature of protein evolution (Somero 1995). When the balance is broken, the denatured proteins induce the upregulation of heat shock proteins (Hsps) (Parsell & Lindquist 1993; Feder & Hofmann 1999), and animals inhabiting different intertidal regions have different expression patterns of heat shock protein against thermal stress (Tomanek & Somero 1999; Dong et al. 2008; Clark & Peck 2009a,b; Dong & Williams 2011). The heat shock response is also an important physiological limit in determining biogeographical range shifts in the scenario of climate change (Tomanek 2008; Somero 2012).

The energy allocation strategies are important for intertidal zonation (Marshall et al. 2011; Sokolova et al. 2012). AMP-activated protein kinase (AMPK) and histone/protein deacetylase Sirtuin1 (SIRT1) are fuel-sensing molecules (Ruderman et al. 2010). SIRT1 regulates fat and glucose metabolism in response to physiological changes in energy levels, thereby acting as a crucial regulator of the network that controls energy homeostasis (Cantó et al. 2009; Houtkooper et al. 2012). Because activation of AMPK and SIRT1 indicates the switching on of catabolic pathways and the switching off of anabolic pathways (Cantó et al. 2009), the upregulation of these 2 metabolic sensors also indicates that more energy is being allocated to maintenance and less energy to growth, storage, and reproduction.

Limpets are common species of rocky intertidal communities from tropical to Polar Regions (Nakano & Ozawa 2007) and occupy different intertidal zones and microhabitats (Shotwell 1950; Haven 1973; Wolcott 1973). Cellana toreuma is distributed widely on the coast of China from Liaoning Province to Hainan Island and occupies mid–low intertidal zones (Morton & Morriston 1983; Williams & Morritt 1995). The cardiac performance of C. toreuma is sensitive to ambient temperature. In summer, when ambient temperature is always beyond the upper limit of cardiac performance, large-scale mortality of C. toreuma populations occurs (Williams & Morritt 1995; Chelazzi et al. 2001; Dong & Williams 2011), especially in tidal pools (Firth & Williams 2009). In the present study, the aerial and aquatic cardiac performances of C. toreuma were determined at different temperatures to investigate the physiological adaptations of this mid-intertidal gastropod to rapid changes and extreme thermal stress. The cardiac performance and expression of biomarkers (Heat shock proteins, AMPK and SIRT1) were also determined in a simulated tidal cycle, to study the physiological adaptations to environmental conditions that changed from fully submersed to fully terrestrial.
MATERIALS AND METHODS

In situ temperature measurement

Robolimpets were used to measure the in situ body temperature of limpets (Lima & Wethey 2009). In this method, a bio-mimetic data logger consists of a micro-logger inserted into the shell of a limpet from which soft tissues have been removed. In the present study, the operative body temperatures of limpets were estimated using Robolimpets at a field site on Nanding Island, Fujian, China (24°09’N, 117°59’E). Three Robolimpets were deployed on a semi-wave-exposed shore in 2.0 m above chart datum (CD, the level of water that charted depths displayed on a nautical chart are measured from), in the range where C. toreuma is known to migrate vertically up and down the shore during a tidal cycle (Y. W. Dong and G. D. Han, unpubl. data). Operative temperature recordings were made every 30 min, and the aeral and aquatic body temperatures were analyzed during both neap tides and spring tides (20 to 31 July 2012).

Animal sampling and maintenance

All C. toreuma were collected from Nanding Island in March 2012. After collection, the specimens were transported back to the State Key Laboratory of Marine Environmental Science of Xiamen University, Xiamen. All limpets were acclimated on a ceramic tile that was used to mimic the natural rocky shore. The ceramic tile was scoured by seawater using a seawater pump in advance to stimulate growth of a bio-film. During the acclimation C. toreuma were sprayed using seawater (20°C) constantly and seawater was replaced daily.

Heart rate assays

The heart rate experiments were carried out by a non-invasive method that was developed by Depledge and Andersen (1990) and modified by Chelazzi et al. (1999). An infrared sensor was attached to the shell close to the heart of each individual with BlueTac (Bostik, UK). The heart rate signals were amplified and transformed with Powerlab (4/30, ADInstruments, Dunedin, New Zealand) to a voltage waveform that could be converted into visual data with Chart (version 5.0).

The heart rate assays were carried out in 3 experiments to measure the heart rates of C. toreuma (body size, 1.8 ± 0.4 cm) in air, water, and a simulated tidal cycle, respectively. The aerial heart rate of C. toreuma was measured at 25, 30, 35 and 40°C (n = 4) as described previously (Dong & Williams 2011) with minor modifications. After acclimation, animals were placed under a seawater spray (approximately 20°C, 30 psu) for 30 min to allow them to replenish mantle water lost during transfer, and then subjected to a simulated “emersion” period. Animals were placed into a beaker, which was immersed in a water bath, allowing the air temperature in the beaker to be increased from 20°C to designated temperatures in 2 h. After being maintained at the designated temperature for 1 h, temperature was decreased to room temperature (18.3°C). Temperatures of the beaker and limpet body temperatures were recorded every minute using a thermometer (Fluke 54II, Fluke, WA, USA). Real-time heart rates were recorded every minute. The ABT, the temperature at which heart rate decreases suddenly and which reflects the thermal limitation of C. toreuma, was determined using regression analyses to generate the best-fit line on both sides of the putative break points (as described by Stillman & Somero 1996).

The aquatic heart rates of C. toreuma were measured at 25, 30, 35 and 40°C (n = 4) similar to the aerial heart rate measurements. After acclimation, animals were placed into beakers with aerated seawater (approximately 20°C, 30 psu). The water temperature was increased to the designated temperatures over 2 h and then maintained for 1 h. Real-time water temperatures and heart rates were recorded every minute. ABT was determined as above.

To determine the change of heart rate during a tidal cycle, heart rates of limpets were recorded every minute in a simulated semi-diurnal tidal cycle for 48 h. The simulated tidal cycle system was composed of a pump, rock tile, a 1000-w lamp and a tank to control the water height and temperature. To adapt to the environmental conditions, 40 individuals were placed in the simulated tidal cycle system for a week before heart rate measurement. In the first 24 h, a simulated semi-diurnal tidal cycle in room temperature (17–20°C) was applied. In order to study the effect of thermal stress on physiological performance, air temperature was increased to 35°C, and then decreased to room temperature (Fig. 1). When limpets were submersed or emersed as shown in Fig. 1, 5 individuals were collected and stored at −80°C to measure gene expressions.

Heat-shock protein assay

Total RNA was isolated from approximately 50 mg of foot muscle using Trizol Reagent (Invitrogen, Carlsbad, CA, USA). The first strand of cDNA was synthesized using total RNA as a template. Reverse transcriptase (RT) reactions were performed using PrimeScript
RT reagent Kit with gDNA Eraser (TAKARA, Shiga, Japan). To obtain sequences of target genes from *C. toreuma*, PCR was used to amplify partial sequences with degenerate primers (the sequences of primers used in this study are given in the supplementary material Table 1), and then the full-length cDNAs were obtained using the rapid-amplification of cDNA ends (RACE) protocol with a 3'-Full RACE Core Set and a 5'-Full RACE Kit (TAKARA, Shiga, Japan). A partial sequence of the \( \beta \)-actin gene was selected as a reference housekeeping gene to normalize the level of expression. The levels of *hsp70*, *hsp90*, *ampk\( \alpha \)*, *ampk\( \beta \)* and *sirt1* expression were quantified using real-time quantitative PCR with primers designed from the sequences obtained as described above (GenBank accession No. *hsp70*, JX69849; *hsp90*, JX69850; *ampk\( \alpha \)*, JX69847; *ampk\( \beta \)*, JX69848; *sirt1*, JX69851). PCR was carried out in an ABI 7500 Real-Time PCR System (Applied Biosystems, MA, USA) in a 20-\( \mu \)L reaction volume containing 10 \( \mu \)L of 2\( \times \)FastStart DNA Universal SYBR Green Master (Roche, Germany), 0.8 \( \mu \)L of each primer (10 nmol/\( \mu \)L), 1 \( \mu \)L of cDNA template and 7.4 \( \mu \)L of RNase-free water. The PCR conditions were as follows: 50°C, 2 min; 95°C, 10 min; 40 cycles of 95°C, 20 s; 59°C, 20 s; and 72°C for 40 s with a final dissociation curve step. All samples were measured in triplicate. Threshold cycle (Ct value), the intersection between an amplification curve and a threshold line, was analyzed using the ABI 7500 System Software (Applied Biosystems, MA, USA). The expression of *hsp70*, *hsp90*, *ampk\( \alpha \)*, *ampk\( \beta \)* and *sirt1* mRNA for the various heat treatments was determined as the relative value of \( \beta \)-actin for experimental against control treatments.

**Statistics**

Data were analyzed using SPSS 15.0 (Chicago, USA). To compare the differences in ABTs among aerial and aquatic conditions, the Mann–Whitney U-test was implemented. The relationship between heart rate and temperature in the simulated tides was analyzed using linear regression. To compare the differences in *hsp70*, *hsp90*, *ampk\( \alpha \)*, *ampk\( \beta \)* and *sirt1* between different time points, one way ANOVA was carried out followed by Duncan’s post hoc pairwise comparisons. Differences were considered significant if \( P < 0.05 \).

**Figure 1** Change of temperature and emersion/submersion in the simulated semi-diurnal tide. Arrows represent the time points for animal collections, and shaded areas show the periods of being submersed.

**Figure 2** (a) Continuous recording of operative body temperature and (b) statistical results for *Cellana toreuma*. Body temperature was measured using Robolimpets that were deployed on the mid-intertidal shore in Nanding Island from 20 to July 30. The bars indicate the maximum and minimum temperatures. The lower and upper ends of the boxes represent the 25 and 75% percentiles, respectively. ‘+’ represents the mean value.
RESULTS

**In situ operative body temperatures of Robolimpets**

From 20 to 31 July 2012, the operative body temperatures of Robolimpets in the mid-intertidal zone changed dramatically in relation to the tidal cycle (Fig. 2). Compared to the body temperatures of submersed animals, body temperatures of emersed animals were more variable, and the thermal regimes in air and underwater were very different.

In water, the body temperature ranged from 24.79 to 28.19°C (mean ± SD, 26.19 ± 0.75°C), and the 25 and 75% percentiles were 25.76 and 26.73°C, respectively. The median was 25.76°C, and the coefficient of variance (CV) was 2.86%.

In air, the body temperature ranged from 23.82 to 48.11°C (mean ± SD, 32.41 ± 6.47°C), and the 25 and 75% percentiles were 27.70 and 36.45°C, respectively. The median was 30.62°C, and CV was 19.98%.

**Heart rates of limpets in air and water**

The heart rates of both submersed and emersed *C. toreuma* were sensitive to ambient temperature (Figs 3 and 4). In water, heart rates increased with increasing temperature from 20 to 30°C. However, heart rate decreased when temperature was over 30°C. The ABT of heart rate in water was 30.2°C (Fig. 5). In air, heart rates also increased with increasing temperature, but the ABT of heart rate in air (34.2°C) was significantly higher than that in water (Mann–Whitney *U*-test, *P* = 0.037).

The ratios of aerial to aquatic heart rates were temperature-dependent, and increased with increasing temperature. The ratios were 1.00, 1.40 and 1.72 at 25, 30 and 35°C, respectively. The temperature coefficients (*Q*₁₀) in the temperature range from 20 to 30°C were 1.56 and 2.19 in water and air, respectively.

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**Figure 3** The heart rates of *Cellana toreuma* submersed at different temperatures (mean ± SD for *n* = 4). The dashed line represents water temperature.
**Figure 4** The heart rates of *Cellana toreuma* emersed at different temperatures (mean ± SD for *n* = 4). The dashed line represents air temperature.

**Table 1** Primers used for real-time polymerase chain reaction amplification

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Objectives</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>qACTINF</td>
<td>GAAGGATGGCTGGAACAA</td>
<td>Real-time primer for β-actin</td>
<td>Self-design</td>
</tr>
<tr>
<td>qACTINR</td>
<td>CCGAGACATCAAGGAGAAG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>qAMPKαF</td>
<td>CGATTGTAGTGTAGATGTGT</td>
<td>Real-time primer for ampkα</td>
<td>Self-design</td>
</tr>
<tr>
<td>qAMPKαR</td>
<td>GCCATTCTCTGATTGTCTAT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>qAMPKβF</td>
<td>AATGTGAATAGTGAACGGATA</td>
<td>Real-time primer for ampkβ</td>
<td>Self-design</td>
</tr>
<tr>
<td>qAMPKβR</td>
<td>AGCATAACAGCAGAACTC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>qHSP70F</td>
<td>AATATAAGGAAGGAGCAGAGAG</td>
<td>Real-time primer for hsp70</td>
<td>Self-design</td>
</tr>
<tr>
<td>qHSP70R</td>
<td>TATCAGCCAGAGCAATTAGC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>qSIRT1F</td>
<td>GCTGCTGATAAGGATGAGG</td>
<td>Real-time primer for sirt1</td>
<td>Self-design</td>
</tr>
<tr>
<td>qSIRT1R</td>
<td>TACATTGGCTGGAAGAGA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>qHSP90F</td>
<td>ATGATCGCTAGTTTGTTG</td>
<td>Real-time primer for hsp90</td>
<td>Self-design</td>
</tr>
<tr>
<td>qHSP90R</td>
<td>AGTTGGCTGGGTCAGGTG</td>
<td></td>
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</tbody>
</table>
Figure 5 Arrhenius breakpoint temperatures (ABT) of heart rates for representative individuals (a) submersed and (b) emersed. The ABT of heart rate of emersed animals (34.2°C) was significantly higher than those submersed (30.2°C).

Figure 6 Variations of heart rates of Cellana toreuma in simulated semi-diurnal tides. Temperature was controlled at approximately 20°C in the first day and increased from 20 to 35°C in 2 h and maintained at 35°C for 1 h at the second day. Solid arrows show transitions between emersed and submersed. The shaded areas show the periods of being submersed.

Figure 7 The relationship between temperature and (a) total heart rate, (b) heart rate of submersed animals and (c) heart rate of emersed animals in the simulated tide.

Physiological adaptations of limpets in simulated tides

Animals were submersed and emersed periodically in the simulated tide, and the heart rates were relatively stable at room temperature. During the entire experimental period, heart rates closely depended on temperature. When temperature increased from approximately 20 to 35°C, heart rates increased from 75 beats/min to
220 beats/min. Generally, linear relationships existed between temperature and heart rates (Fig. 7). The slopes of the regressions of total heart rate, heart rate in water and heart rate in air were 7.63 ($R^2 = 0.80$), 4.567 ($R^2 = 0.301$) and 7.63 ($R^2 = 0.807$), respectively.

The levels of hsp70 and hsp90 mRNA were relatively stable in transitions between submersion and emersion (Fig. 8). However, thermal stress induced the up-regulation of hsps significantly (1-way ANOVA, $F_{(7, 34)} = 6.715$, $P < 0.001$ for hsp70; $F_{(7, 34)} = 6.715$, $P = 0.032$ for hsp90).

The levels of ampka, ampkb and sirt1 were also stable in the transitions between submersion and emersion at all time points (Fig. 9). One-way ANOVA results showed there were not significant differences in gene levels among different time points ($F_{(7, 36)} = 0.570$; $P = 0.775$ for ampka; $F_{(7, 34)} = 0.857$; $P = 0.552$ for ampkb; $F_{(7, 36)} = 0.927$; $P = 0.501$ for sirt1).

**Figure 8** Expression of (a) hsp70 and (b) hsp90 of Cellana toreuma at the time points in the transition between submersed/emersed as shown in Figure 1. Values with different letters are significantly different ($P < 0.05$) among different time points.

**Figure 9** Expression of (a) ampka, (b) ampkb and (c) sirt1 of Cellana toreuma at the time points in the transition between submersed/emersed as shown in Fig. 1. There were no significant differences ($P > 0.05$) among different time points.
DISCUSSION

The pattern of cardiac performance in relation to temperature is an adaptive physiological response to the ambient thermal regime in the habitat. In the intertidal zone, animals experience daily transition from submersion to emersion, and then have to adapt to the different thermal regimes in air or in water. The percent emersion time of intertidal molluscs varies in relation to zonation and the percentage of emersion time in mid-intertidal zone is from 14 to 86% (Newell 1979), with differences in the coastlines of different continents (Finke et al. 2007). In the present study, _C. toreuma_ is a typical mid-intertidal species and is exposed to air twice daily due to the semi-diurnal tide at the study site (Nanding Island in East China Sea). From 22 to 30 July 2012, the air exposure percentage of Robolimpets, which were deployed in the distribution zone of the live animals, was 41.6%. In addition to the change of emersion and submersion, the thermal regimes in air and in water were dramatically different. Based on our 1-year-round observation of operative body temperatures, July was the hottest season in 2012 at the study site. In air, the maximum temperature in the mid-intertidal zone was over 48°C, and the temperature increased from approximately 26 to 48°C with a rate of >4°C·h⁻¹. However, the temperature was relatively stable in water and there was no large daily variation. Therefore, limpets experienced rapid changes and extremes of thermal stress during emersion.

The different patterns of aerial and aquatic cardiac performances in relation to temperature are adaptive responses to the ambient environmental conditions. The respiratory response to emersion is related to the duration of aerial exposure. In the present study, the ratio of the aerial : aquatic heart rate was 1:1 at 25°C, and the ratio was similar to the ratio of the aerial : aquatic oxygen consumption rate for most mid-intertidal gastropods (McMahon 1988). When temperature increased, the ratios of the aerial : aquatic heart rate kept increasing from 20 to 30°C resulting from the fast enhancement of aerial heart rate (aerial Q₁₀: 2.19; aquatic Q₁₀: 1.56). This result can also explain previous contrasting results about the oxygen consumption between emersed and submersed limpets (Santini et al. 2000) because the ratio of the aerial : aquatic heart rate is temperature-dependent in this intertidal limpet.

Animals emersed can tolerate higher temperatures than those submersed in the present study. The upper thermal limits in emersed and submersed limpet are different, and the emersed limpets showed higher ABT (34.2°C) than the submersed animals (30.2°C). When heated to 40°C, 50% of submersed animals died, and all emersed animals survived. As mentioned above, the air temperature in the mid-intertidal zone could frequently exceed 40°C and was much higher than water temperature in summer. The differences in ABTs and upper limits should be an adaptation to the rapid changes and high air temperatures, and are crucial for the limpet against the high air temperature. This result can also partly explain the high mortality of _C. toreuma_ in tidal pools in summer observed by Firth and Williams (2009) and our personal observations.

The recovery of heart rates after thermal stress between submersed and emersed limpets showed similar patterns (Figs 4 and 5). The heart rates returned to the original levels when temperature decreased to the room temperature. These results indicated that submersed and emersed limpets could efficiently use both dissolved oxygen and atmospheric oxygen, respectively. In air, the gastropod adaptations to emersion are reduction of the ctenidium surface area and formation of a mantle cavity lung (McMahon 1988). In the present study, there was no obvious increase of heart rate during recovery from thermal stress (Figs 3–7), indicating the absence of anaerobic metabolism during thermal stress and repayment of oxygen debt during recovery. However, these results about oxygen debt need be confirmed in further studies.

In the simulated tidal cycle, the heart rates were relatively stable and temperature-dependent. However, no significant relationship between heart rate and temperature was found in submersed limpets, probably due to the narrow thermal range of the water (Santini et al. 2000). Limpets become active when they are submersed. The change in behavior can affect the relationship between heart rate and temperature because active limpets have higher heart rates than those of resting animals (Santini et al. 2000). During the transitions between emersion and submersion, there was no obvious change of heart rate at room temperature, and this further confirms that the ratio of the aerial : aquatic heart rate is approximately 1 at room temperature (approximately 20°C), and that _C. toreuma_ can effectively use atmospheric oxygen as an adaptation to the mid-intertidal habitat.

Desiccation can induce the upregulation of _hsp_ in various organisms (reviewed in Feder & Hofmann 1999; Sorensen et al. 2003). In the present study, however, aerial exposure to room temperature did not induce the up-regulation of heat shock proteins in _C. toreuma_ during
the simulated tidal cycle except 6 h after thermal stress. The levels of \textit{ampk\textalpha}, \textit{ampk\textbeta} and \textit{sirt1} during the tidal cycle were also relatively stable, even after thermal stress. The low levels of the 3 metabolic sensors after thermal stress could be because the temperature of 35°C is beyond the thermal window of gene expression (Han et al. 2013). Overall, the patterns of molecular markers indicate that there is no obvious protein damage and change of ratio of ADP/ATP during transitions from emersion and submergence at room temperature. However, high temperature can lead to protein denaturation in the species, and the upregulation of \textit{hsp} at time “G” in Figure 8 is similar to our previous observations (Dong et al. 2008; Dong & Williams 2011).

In summary, the mid-intertidal limpet \textit{C. toreuma} has different physiological adaptive strategies to aerial and aquatic thermal regimes. Compared to that of the submersed animals, the higher temperature coefficient (Q_{10}), higher ABT of emersed animals and increasing ratio of aerial : aquatic heart rates with increasing temperature indicate that this species can effectively utilize atmospheric oxygen, and the species is adapted to the rapid change and extreme thermal stress in air. However, the \textit{in situ} operative body temperature frequently exceeds the aerial ABT of \textit{C. toreuma}, providing an explanation for the occurrence of large-scale mortality of \textit{C. toreuma} in summer. In the scenario of climate change, this species will be sensitive to increasing temperature, and local extinction could possibly occur on subtropical shores.

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