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# A simulated heat wave shortens the telomere length and lifespan of a desert lizard

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ABSTRACT

Understanding how organisms respond to warming contributes important information to the conservation of biodiversity that is threatened by climate warming. Here, we conducted experiments on a desert agama (*Phrynocephalus przewalskii*) to test the hypothesis that climate warming (an increase in both mean temperature and heat waves) would induce oxidative stress, shortening telomere length, and thereby decreasing survival. Our results demonstrated that one week of exposure to a simulated heat wave significantly shortened telomere length, and decreased the overwinter survival of lizards, but mean temperature increase did not affect the survival of lizards. However, the antioxidant capacity (anti-oxidative enzyme) was not affected by the warming treatments. Therefore, heat waves might have negative impacts on the desert agama, with shortened telomeres likely causing the lifespan of lizards to decrease under climate warming.

## 1. Introduction

Ongoing climate warming is enhancing the global mean surface temperature and increasing temperature variability, with more hot extremes occurring (i.e., heat wave) (IPCC, 2013; Thornton et al., 2014), which has pervasive and profound impacts on the life of organisms and ecosystems they use (Thomas et al., 2004; Barange et al., 2014). Reptiles are especially vulnerable to climate warming because their behaviour, physiology, and life history are highly dependent on environmental temperature (Deutsch et al., 2008; Sinervo et al., 2010; Huey et al., 2012). A recent study showed that lizards might grow fast, but die young, in the context of climate warming (Bestion et al., 2015). However, the proximate mechanisms underlying the shortened lifespan of lizards have not been explicitly revealed. One such proximate mechanism might involve the disturbance of balance between the oxidation and antioxidant defence systems that causes oxidative stress. For instance, heat exposure induces oxidative stress that shortens the length of telomeres, which are complex DNA-protein caps of eukaryotic chromosomes that function to maintain genome integrity (von Zglinicki, 2002), shortened telomere length might be associated with ageing and therefore the survival and lifespan of animals, although this relationship is not uniform across taxa (Finkel and Holbrook, 2000; Monaghan, 2010; Banh et al., 2016).

Global drylands have experienced, and will continue to face, much more severe increases in temperature than humid areas (Huang et al., 2017). Lizards that inhabit drylands are especially sensitive to climate warming, due to sparse vegetation cover, which provides limited opportunities for thermoregulation to cool down their bodies (Kearney et al., 2009). In this study, we conducted a warming experiment to determine how an increase in mean temperature and heat waves influences the survival rate, oxidative stress, and telomere length of the desert toad-headed agama, *Phrynocephalus przewalskii*. In particular, we aimed to test the hypothesis that an increase in mean temperature and heat waves induces oxidative stress that might shorten telomere length and cause the survival (and, therefore, lifespan) of lizards to decrease.

## 2. Materials and methods

### 2.1. Study species

The desert toad-headed agama (*Phrynocephalus przewalskii*) is a small lizard species [44–56 mm adult snout-vent length (SVL)] that

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inhabits arid and semi-arid regions (Zhao et al., 1999). The preferred body temperature, critical thermal maximum ($CT_{max}$), and critical thermal minimum ($CT_{min}$) of this species are 36.6 °C, 47.1 °C and 1.3 °C, respectively (Qu et al., 2011; Li et al., 2017). In summer, the operative temperatures ($T_e$s) differ among microhabitats, ranging from 28.9 ± 1.3 °C in full shade to 41.2 ± 1.3 °C in full sun. Furthermore, field body temperatures fluctuate with the time of day, with a mean body temperature of 38.1 ± 0.2 °C during the daytime period from 09:00–17:00 (Li et al., 2017).

### 2.2. Experimental design and thermal treatments

To simulate the thermal environment of lizards with greater precision and to design the thermal regimes for the warming experiments, we determined the field body temperature of lizards in August 2012 and 2013. We captured adult *P. przewalskii* by hand at our field site from 08:00 to 18:00, and the body temperature of the captured lizards was measured immediately ( ± 0.1 °C) by inserting the probe of the UT325 electronic thermal meter (Shenzhen Meter Instruments, Shenzhen, China) into the cloaca (about 5 mm). The body temperature of the lizards at night (from 19:00 to 07:00) was estimated from the ambient temperature of the burrows where they hide at night. The burrow temperatures were recorded by iButtons (DS1921, MAXIM Integrated Products Ltd., USA). Accordingly, the daily body temperature was 32 ± 8 °C (range: 24.8–39.5 °C; Fig. A.1). This information formed the basis of the temperature treatment of the control group in this study. In addition, by the end of the current century, the global mean surface temperature is predicted to increase 0.3–4.8 °C, depending on various global emissions scenarios (IPCC, 2013).

Based on this information, we designed three thermal regimes for the warming experiment: control, warming, and heat wave groups. These regimes simulated the thermal environment experienced by lizards under the current climate, climate warming of 3 °C, and climate warming of 3 °C accompanied by a heat wave of one-week, respectively. The lizards in the control and warming groups were kept at 32 ± 6 °C and 35 ± 6 °C throughout the experiment (14 days), whereas lizards in the heat wave group were primarily maintained at 32 ± 6 °C, and at 38 ± 6 °C for seven days (day 5–11) (Fig. 1). Accordingly, the lizards in the warming and heat wave groups experienced a similar higher (3 °C) temperature (but with different hot temperature extremes) than the lizards in the control group. The experiment was conducted in three programmable incubators (Binder KB 240, Binder GmbH, Tuttlingen, Germany) in which the daily temperatures were set to the regimes of 32 ± 6 °C, 35 ± 6 °C, and 38 ± 6 °C, respectively.

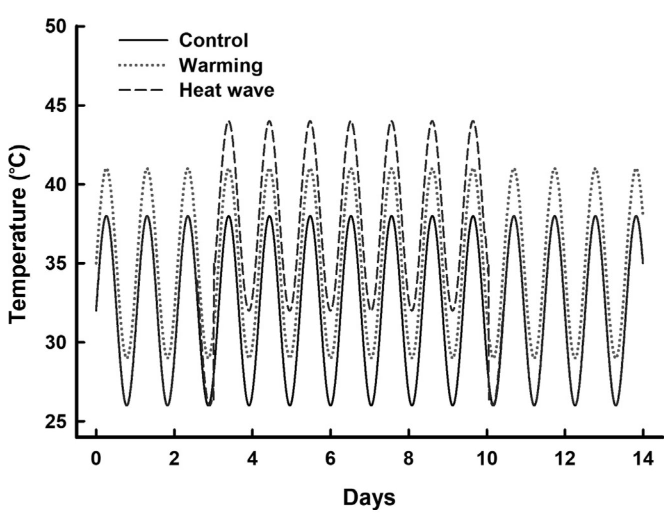


**Fig. 1.** Three thermal treatments used in the present study. The temperature regime of the control group (solid line) was 32 ± 6 °C, mimicking the daily variation of lizard body temperature in the field. The temperature regime of the warming group (red dotted line) was 35 ± 6 °C, mimicking the warming scenario of a 3 °C future increase in the mean temperature. The temperature regime of the heat wave group (blue dotted line) was 32 ± 6 °C, but with seven days (day 5–11) of 38 ± 6 °C, mimicking the hot extremes that are expected to occur during climate warming.

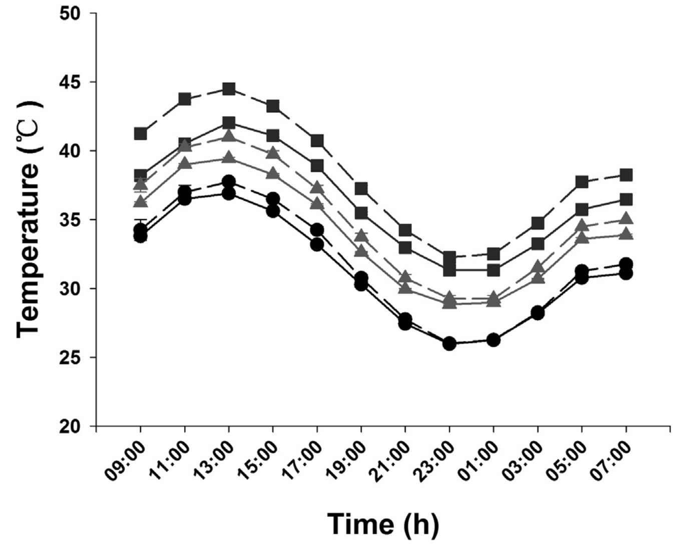


**Fig. 2.** The diel variation of ambient temperatures and body temperatures of *Phrynocephalus przewalskii* lizards under the three thermal treatments of 32 ± 6 °C (•), 35 ± 6 °C (▲), and 38 ± 6 °C (■). —— body temperature, ——— ambient temperature.

In late September 2015 (post-breeding season), we collected 88 adult *P. przewalskii* (male:female = 49:39) from Jungar Banner, Inner Mongolia, China (40° 12′ N, 111° 07′ E; elevation 1036 m). The lizards were transported to our laboratory in Beijing, where they were kept in three containers (600 × 430 × 340 mm) within a climate controlled room with a temperature of 24 °C and a photoperiod of 14:10 (L:D). To produce a thermal gradient for lizard thermoregulation, a full-spectrum bulb (25 W) was set at one end of all containers to provide heating source from 09:00 to 16:00 every day. For all individuals, snout–vent length (SVL) was measured to 0.01 mm, and body mass (BM) was weighed to 0.001 g by using a vernier caliper (Kanon Instruments, Japan) and an electronic balance (Mettler-Toledo GmbH, Greifensee, Switzerland). After one-week of acclimation, these lizards were allocated randomly to three temperature treatments [control (n = 28, M:F = 16:12), warming (n = 30, M:F = 16:14), and heat wave (n = 30, M:F = 17:13)] to evaluate how experimental warming affects the oxidative stress and survival of lizards. In each treatment, 8–10 lizards were kept in each of three containers (600 × 430 × 340 mm). The bottom of each container was filled with 5 cm-thick sand to mimic the habitat usually occupied by the lizards. The lizards buried themselves in sand for thermoregulation. The photoperiod was 14:10 (L:D). Food (crickets, *Acheta domesticus*, dusted with mixed vitamins and minerals) and water were provided ad libitum. On the fifth day of the warming experiment, we measured the cloacal temperatures of 30 lizards using an electronic thermometer (UNT325, Unitrend electrical limited Liability Company, Shanghai, China). The body temperatures of lizards were 31 ± 0.3 °C, 34 ± 0.3 °C and 36 ± 0.3 °C when they were exposed to thermal regimes of 32 ± 6 °C, 35 ± 6 °C, and 38 ± 6 °C respectively (Fig. 2).