

## LETTER

# Recurrent sublethal warming reduces embryonic survival, inhibits juvenile growth, and alters species distribution projections under climate change

**Abstract**

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**INTRODUCTION**

For organisms with complex life cycles, ecological consequences of climate change may be driven by responses to warming that vary across ontogeny (Kingsolver *et al.* 2011; Radchuk *et al.* 2013). With rapid warming, a major goal for ecologists is to determine thermally sensitive processes that underlie shifts in range dynamics (Pacifici *et al.* 2015; Urban *et al.* 2016). Recent advances in species distribution models (SDMs) incorporate biological mechanisms to predict climate-driven range shifts (Helmuth *et al.* 2005; Buckley *et al.* 2010; Riddell *et al.* 2017) but often rely upon adult life stages to make predictions (e.g., Sykes *et al.* 1996; Buckley 2008; Deutsch *et al.* 2008; Randin *et al.* 2009; Kearney 2013). Downstream effects from early life have consequences for growth, survival, and reproduction (reviews in Lindström 1999; Podolsky & Moran 2006; Harrison *et al.* 2011). Thus, ecological projections might hinge on responses across ontogeny for many species (Lindström 1999; De Block & Stoks 2005).

Sensitive stages of early ontogeny drive ecological responses to environmental change (Radchuk *et al.* 2013). Sessile stages are sensitive to fluctuating conditions due to limited behaviours and the small range of microclimatic conditions experienced over small spatial extents (e.g., an egg; Refsnider & Janzen 2010; Telemeco *et al.* 2016; but see Du & Shine 2015). Embryos consequently rely on physiological responses to developmental conditions that can alter growth and development rates and increase mortality (e.g., Castro *et al.* 2005; Georges *et al.* 2005; Hepp *et al.* 2006; Oufiero & Angilletta 2006; Potter *et al.* 2011). In turn, developmental conditions may influence population dynamics through changes in maturation rates, reproductive success, and survival (e.g., Haywood

& Perrins 1992; Lumey & Stein 1997; Warner & Andrews 2002; DuRant *et al.* 2010; Larios *et al.* 2014), particularly in short-lived species (Tinkle 1969; Overall 1994). Downstream effects of warming also increase risk of extirpation by reducing reproductive performance and survival (Edmunds 2005; Neilson *et al.* 2005; Crozier *et al.* 2008). Impacts of thermal fluctuations in early ontogeny should thus be considered in the development of physiologically explicit models (Levy *et al.* 2015; Urban *et al.* 2016).

The lasting effects of warming during early ontogeny may be underestimated by ignoring impacts of fluctuating thermal conditions. Recurrent sublethal stressors – exposures to sub-optimal conditions that are not acutely lethal – are increasingly likely as climate warming increases daily temperature variance and frequencies of extreme weather events (Meehl & Tebaldi 2004; IPCC 2013). Modest increases in temperature can benefit growth and development (Angilletta *et al.* 2004b; Refsnider & Janzen 2010), particularly in environments where low temperatures limit growth (Deutsch *et al.* 2008; Randin *et al.* 2009; Paaijmans *et al.* 2013). However, in warmer environments, increased incubation temperatures may result in recurrent sublethal extremes that lead to chronic stress (Campbell *et al.* 1998; Badyaev 2005), which can inhibit development, increase embryo mortality and influence lifetime fitness (e.g., Shine & Elphick 2001; Fly & Hilbish 2013; Marshall & Sinclair 2015). Recent SDMs incorporate ontogenetic variation of thermotolerance for some well-studied species (e.g., Crozier *et al.* 2008; Levy *et al.* 2015). Clearly, lethal thresholds influence fitness; however, physiologically-explicit SDMs based solely on lethal limits ignore consequences of recurrent sublethal fluctuations (Woodin *et al.* 2013). Unfortunately, the preponderance of constant-temperature treatments in physiological studies has left little focus on

fluctuating developmental regimes (Niehaus *et al.* 2012; Bowden *et al.* 2014). Constant incubation temperatures have advanced research by elucidating thermal sensitivities of phenotypes across many oviparous taxa (reviews in Deeming & Ferguson 1991a; Booth 2006; Bowden *et al.* 2014). However, the applicability of that data to development under natural conditions is limited. By overlooking acute and recurrent thermal stressors, incubation under constant temperatures poorly predicts development under natural cycles (reviews in Bowden *et al.* 2014; Warner 2014; Wu *et al.* 2015). Thermal stress on anurans and *Manduca sexta* larvae reared under constant temperatures resulted in reaction norms that poorly predicted growth and development under naturalistic regimes (Niehaus *et al.* 2012; Kingsolver *et al.* 2015). Thermal impacts on development underscore the importance of experimental conditions for the embryonic environment.

Here, we use naturalistic thermal cycles to examine consequences of recurrent sublethal warming during incubation on embryonic and post-hatching phenotypes. We integrate these findings to predict the species distribution of *Sceloporus undulatus*, a widespread North American lizard. Maternal behaviour of *S. undulatus* suggest that females nest in the warmest parts of their environment, digging shallow nests where embryos experience diel thermal cycles (Fig. 1a,b; Angilletta *et al.* 2000, 2009). Increases in temperature means and variances of *Sceloporus* embryos can speed growth and development without affecting survival (e.g., Sexton & Marion 1974; Andrews *et al.* 2000; Angilletta *et al.* 2000; Oufiero & Angilletta 2006). However, our study is the first to warm embryos throughout incubation beyond regimes experienced at contemporary nest sites in this system. In the laboratory, we reared embryos under treatments that simulated contemporary and potential future thermal conditions. In a complementary field experiment, we artificially warmed natural nests to simulate similar sublethal warming. We integrated embryonic responses to warming into an SDM using a life-cycle submodel of population dynamics (Levy *et al.* 2015). Model projections indicate that moderate warming during early ontogeny can limit species ranges. Our study highlights consequences of transient, but recurrent, exposure to warmer nests that may harm embryos and hatchlings, shaping ecological responses to environmental change.

## METHODS

### Laboratory methods

#### *Collection & husbandry*

To examine impacts of sublethal warming during incubation, we conducted experiments using *S. undulatus* eggs from females collected in Edgefield County, South Carolina (SC) in May and June 2014 (UTM Easting 396467.43, Northing 3753517.85, Zone 17S). We housed adult lizards at Clemson University in terraria (8.48 L; 30 × 19.5 × 14.5 cm) with moist sphagnum for oviposition. Programmable environmental chambers (I-36VL; Percival Scientific, Perry, IN, USA) maintained 14:10-hour light:dark cycles and kept lizards at preferred daytime (32 °C) and approximate nighttime (24 °C) temperatures (Niewiarowski 1992; Angilletta 2001). We

replenished water daily and offered crickets *ad libitum* every 2 days.

Collection and care of eggs minimised exposure to conditions outside treatment designs. We checked terraria hourly 0700–2100 to immediately weigh and place eggs in individual containers (59 mL; 3 cm-height-by-5 cm-diameter) with a 1:100 water-to-silica-sand mixture (Angilletta *et al.* 2000). Environmental chambers (I-36VL; Percival Scientific) maintained eggs at 80% relative humidity and temperatures per treatment designs. We replaced water lost from containers every 3 days to maintain hydric conditions throughout incubation. We rotated treatment groups between chambers and rotated shelves in a balanced randomised design to control for potential effects of chamber or shelf location. Hatchlings were transferred to containers (474 mL; 7.5 cm-height-by-9 cm-diameter) under the same conditions as adults, except pinhead crickets were offered daily.

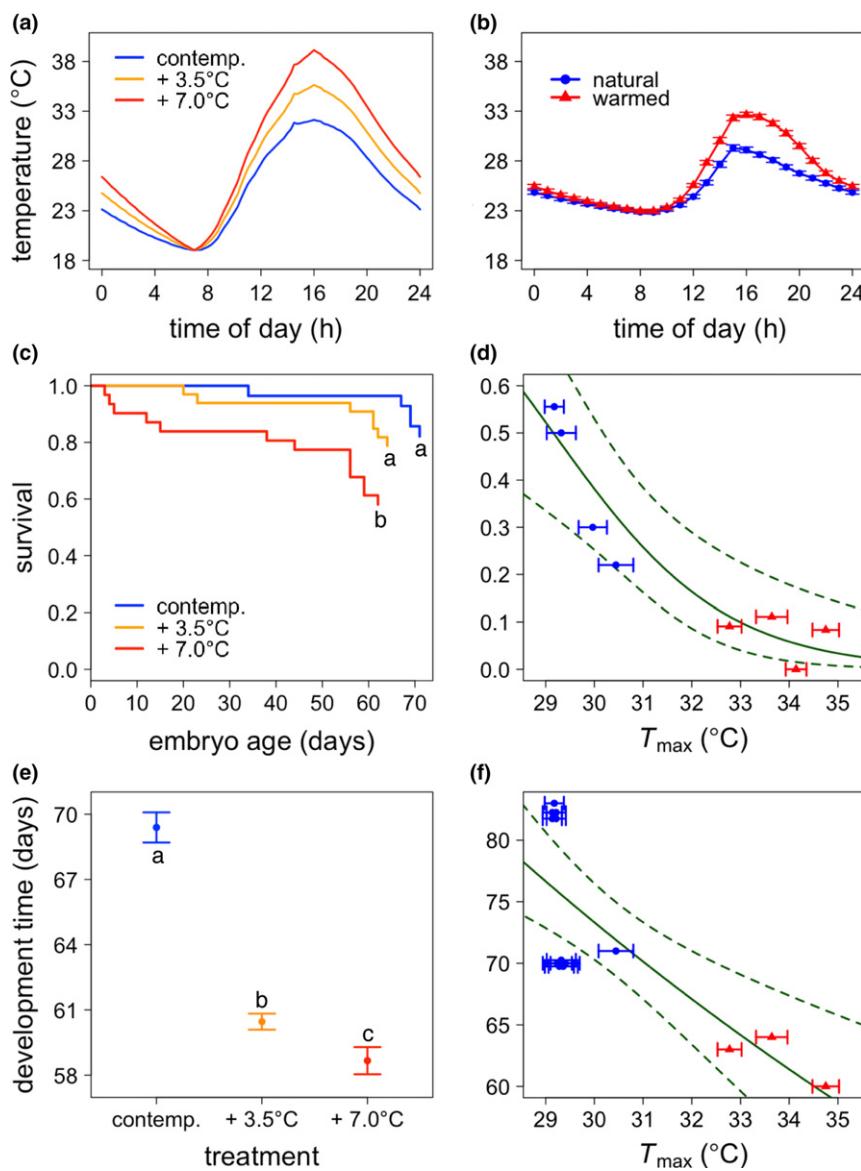
#### *Treatment design*

We designed the treatment to create naturalistic thermal regimes based on soil temperatures recorded in simulated nests in Edgefield County, SC (Angilletta & Sears, unpublished data), which were constructed assuming nesting conditions consistent with those observed by Angilletta *et al.* (2009). The treatments included a thermal regime that estimated contemporary SC nest temperatures and two regimes that increased daily maximum temperature ( $T_{\max}$ ) to simulate warming scenarios (Fig. 1a). Angilletta *et al.* (2013) suggested that exposure to high  $T_{\max}$  was not necessarily harmful to *S. undulatus* embryos below a lethal threshold (~41 °C). However, they measured effects of acute exposure. To examine impacts of recurrent exposures to high  $T_{\max}$  throughout incubation, we increased  $T_{\max}$  in the warming treatments by 3.5 and 7.0 °C relative to the contemporary treatment (32.0 °C). Thus, embryo  $T_{\max}$  increased to suboptimal levels without reaching the lethal threshold. Though climate warming also increases nighttime minima (Donat & Alexander 2012; IPCC 2013), we held daily minimum temperature ( $T_{\min}$ ) at 19.0 °C across treatments to specifically examine effects of increasing  $T_{\max}$ . From 12 clutches [clutch size  $7.67 \pm 0.39$  (SEM), range 6–10], 29 embryos were reared under the contemporary treatment, 33 under +3.5 °C, and 31 under +7.0 °C.

To control for maternal effects, we randomly distributed each clutch evenly among treatments. In *S. undulatus*, oviposition occurs at about 18–26% of embryonic development (Sexton & Marion 1974; Parker *et al.* 2004). We maintained females under common conditions in the laboratory. So, assuming females maintained similar field body temperatures ( $T_b$ ), embryos experienced the same temperatures *in utero*. Therefore, embryos were exposed to maternal  $T_b$  during the earliest stages of embryogenesis and to experimental temperatures during mid-to-late-development.

#### *Embryonic survival & hatchling growth*

We monitored survival daily by checking for heart rates, using an infrared sensor (Buddy Egg Monitor; Avtronics, Cornwall, UK). If no heart rate was detected for three consecutive days, we marked the embryo as deceased on the first day. We measured hatchling mass to 0.1 mg and snout-vent length (SVL)



**Figure 1** Thermal treatment in laboratory and field experiments and impacts of treatments on embryo development time and survival. Error bars indicate  $\pm 1$  SE. (a) Laboratory treatments simulated contemporary thermal conditions at *Sceloporus undulatus* nest sites and warming scenarios designed to introduce recurrent sublethal thermal stressors via increased  $T_{max}$ . (b) In the field, the warming treatment induced sublethal warming of daytime nest temperatures without altering overnight minima. Recurrent sublethal warming reduced embryonic survival in (c) the laboratory and (d) the field. Among lizards that survived to hatching, development time (days from oviposition to hatching) decreased with increased warming in (e) the laboratory and (f) the field. For panels c and e, letters denote statistical relationships such that data with different letters are significantly different ( $P < 0.05$ ). In panel f, overlapping points are offset. See Table 1 for summary statistics.

to 0.1 mm. We then calculated scaled mass indices (SMI) from standard regressions of mass-to-SVL as outlined in Peig & Green (2009, 2010) to estimate hatchling body conditions. We chose SMI as a less biased measure than other indices (e.g., Fulton's index: mass  $\times$  length $^{-3}$ ) that do not account for changing allometry across growth stages (see Appendix S1 for details).

To examine downstream effects of warming treatments, we calculated juvenile growth rates. We repeated body size measurements for the first 3 weeks post-hatching. Then, we used the approach described by Dunham (1978) and Schoener & Schoener (1978) to estimate characteristic growth rates ( $r$ ) for

the interval form of von Bertalanffy growth models. We used SVL instead of mass to minimise potential variation due to nutritional state (Dunham 1978; Sears 2005). We fitted the growth model using Levenberg-Marquardt nonlinear least-squares regression from the *minpack.lm* library in R (Elzhov *et al.* 2015).

## Field methods

### Tracking & collection

In May and June 2015, we tracked gravid females using radio telemetry to locate nests. We attached radio transmitters

(Model BD-2X; Holohil Systems Ltd., Carp, ON, Canada) weighing < 5% of a female's body mass to the dorsum with surgical adhesive. We located eight nests (82 eggs, clutch size  $10.2 \pm 0.36$ , range 9–12) and assigned clutches laid within 5 days of each other to nesting groups, within which we reciprocally transplanted eggs to control for maternal effects. We carefully excavated eggs and placed them in individual containers as in the laboratory methods for transport to Clemson University. We incubated eggs at 15 °C for up to 5 days to allow collection of multiple clutches. This method suspends development without affecting growth and survival after development resumes (Christian *et al.* 1986; Andrews *et al.* 1997). We then reconstructed nests to contain a random sample, including at least one egg from each clutch in the nesting group and totaling the original clutch size laid in that nest. iButton loggers (DS1922L; Maxim Integrated, San Jose, CA, USA) recorded hourly temperatures at mean nest depth.

#### Treatment design

We randomly assigned half the nests to a warming treatment, for which a 0.09 m<sup>2</sup> section of black thermoplastic (Terra-TexSF-D; Hanes Geo, Winston-Salem, NC, USA) was stapled against the soil surface to decrease solar reflectance. There were 44 embryos among the natural nests and 38 among warmed. The material consisted of woven 2.0 mm-wide-by-0.15 mm-thick polypropylene filaments, forming a porous surface that increased daytime nest temperatures without retaining excess heat overnight and allowed for water and gas exchange. To ensure this method did not influence soil moisture or oxygen availability, we performed a validation experiment in which we measured soil temperatures, moisture, and oxygen in a grid of mock nests randomly assigned to the warmed or natural treatment (see Appendix S1 and Table S1 for details).

#### Embryonic survival & hatchling size

We monitored nests daily for emerging hatchlings. Steel wire cages with 3.0 mm spacing placed over nests enabled collection. We calculated survival by counting hatchlings and confirmed results through excavation to count non-viable eggs and empty shells. We measured hatchling mass and SVL and calculated SMI as described above.

#### Data analysis

We conducted statistical analyses in R v3.3.1 (R Core Team 2016). To test effects of laboratory warming treatments on embryonic survival, we used a Cox proportional hazard model from the *survival* library (Therneau 2014), which included an estimator of variance attributable to maternal identity to control for correlation of responses among siblings. To test effects of laboratory treatments on development time, hatchling sizes, SMI, and *r*, we constructed linear mixed effects (LME) models using the *lme* function (Pinheiro *et al.* 2016) with treatment as a categorical variable and maternal identity as a random effect. We added hatchling SVL as a continuous variable for *r* and initial egg mass as a continuous variable for development time and hatchling sizes. For the field data, we constructed LME models with treatment as a categorical

variable and with assigned nest and nesting group as random effects for *T*<sub>max</sub>, *T*<sub>min</sub>, embryonic survival, development time, hatchling body sizes and SMI. We could not include maternal identity in analyses of field data due to the reciprocal transplants. For each parameter in an LME model, we calculated effect sizes ( $\omega^2$ ) to determine the proportion of explained variance of each parameter included in an ANOVA (Olejnik & Algina 2003):

$$\omega^2 = \frac{(SS_{\text{treatment}} - (df_{\text{treatment}} \cdot MS_{\text{error}}))}{(SS_{\text{total}} + MS_{\text{error}})}, \quad (1)$$

where  $SS_{\text{treatment}}$  = sum of squares,  $df_{\text{treatment}}$  = degrees of freedom,  $MS_{\text{error}}$  = mean square error, and  $SS_{\text{total}}$  = total sum of squares.

#### Life-cycle model of population dynamics

##### Modelling embryonic and juvenile survival

We developed an SDM to explore how inclusion of our results affects projections of embryonic survival and population growth in North America. Our model was based on a population dynamic model developed by Buckley (2008) to incorporate biology of free-living *Sceloporus* life stages into population growth projections under climate change and extended to include embryonic development and juvenile survival as in Levy *et al.* (2016b). Parameterization followed previous simulations, except where noted below.

We simulated activity by predicting *T*<sub>b</sub> for female lizards of average size (10.7 g; Angilletta 2001) across the geographic range on surfaces with 0–100% shade. We calculated *T*<sub>b</sub> from operative temperatures (steady state temperature in a microclimate; Bakken 1992), using hourly microclimates (Levy *et al.* 2016a) covering the USA at 36 × 36-km resolution for the past (1980–2000) and future (2080–2100, assuming radiative forcing of +8.5 W m<sup>-2</sup> at year 2100). See Table S2 and Appendix S1 for parameter values and additional details. We assumed that lizards are active when *T*<sub>b</sub> falls within the preferred range (central 80% of field body temperatures; Table S2) and that reproductive season begins after temperatures enable 30 days of activity (Tinkle & Ballinger 1972; Angilletta 2001). On each day of the reproductive season, we simulated oviposition by allocating nests to microhabitats with each combination of shade (0, 25, 50, 75, or 100%) and depth (3, 6, 9, or 12 cm), which captured the range of microhabitats for natural nests (Angilletta *et al.* 2009; this manuscript).

Based on our empirical observations, we evaluated the impacts of warming nest temperatures on embryonic survival and population growth rates by comparing results of the model with and without effects of sublethal warming. We parameterised embryonic survival in the sublethal model using our laboratory survivorship results to provide conservative estimates based on experiments in which we controlled hydric conditions across treatments to isolate the impacts of incubation temperatures. See Appendix S1 for further details.

##### Modelling population growth

We computed population growth rates (*r*<sub>0</sub>, lizards per day) per Buckley (2008):

$$r_0 = m \cdot e_{\text{net}} - \mu, \quad (2)$$

where  $e_{\text{net}}$  = net energy gain by an adult,  $\mu$  = daily mortality ( $197.36 \times 10^{-5}$  lizards per day; Buckley 2008), and  $m$  = eggs produced per Joule ( $3.2 \times 10^{-4}$  eggs per J; Buckley 2008) multiplied by probability of surviving to adulthood. Net energy gain was estimated as the difference between energy gained from feeding and digestion and energy expended during resting and activity. For each location, we calculated the survival to adulthood component of  $m$  as the product of embryonic and juvenile survivorships (Levy *et al.* 2015). We then compared projections of population growth with and without effects of sublethal warming. See Appendix S1 for additional information.

To test how exposure of embryos to recurrent sublethal warming may alter projections through effects on later life stages, we ran the model with different hatchlings sizes and juvenile growth rates to calculate time to maturity. Assumptions built into the model – juvenile survivorship, juvenile growth, and size at maturity do not vary across geography, and all lizards mature by the next reproductive cycle – prevent incorporation of predicted time to maturity into projections. So, we estimated changes in intrinsic growth rates due to delayed maturity using life tables for northern [New Jersey (NJ)] and southern (SC) populations. See Appendix S1 for details.

## RESULTS

### Laboratory & field experiments

The field warming treatment increased  $T_{\max}$  among warmed nests by  $4.21 \pm 0.26^\circ\text{C}$  compared to natural nests and did not alter  $T_{\min}$  across treatments (Fig. 1b, Table 1). We used degree-day calculations (Zalom *et al.* 1983) to compare the magnitudes of warming experienced by embryos due to changes in means and variances between treatments in both experiments (see Appendix S1 for details). Embryos under laboratory warming treatments accrued averages of 257.87 and 336.65 degree-days above the  $T_{\max}$  of the contemporary treatment. In the field, embryos under the warming treatment accrued an average of 309.99 degree-days above the mean  $T_{\max}$  of natural nests. Although absolute temperatures differed between experiments, the field warming treatment induced a magnitude of warming similar to that applied in the laboratory.

Recurrent sublethal warming increased embryonic mortality in both experiments. In the laboratory, embryonic survival decreased with increased warming (Fig. 1c). The proportional hazard model estimated 82.1% survival for the contemporary treatment versus 78.8% for  $+3.5^\circ\text{C}$  and 58.1% for  $+7.0^\circ\text{C}$ . Embryos in the  $+7.0^\circ\text{C}$  treatment had lower survival probability than both the contemporary ( $\beta = -2.84 \pm 1.05$ ,  $z = 2.81$ ,  $P = 0.005$ ) and  $+3.5^\circ\text{C}$  ( $\beta = -1.01 \pm 0.47$ ,  $z = 2.12$ ,  $P = 0.034$ ) treatments. Though survivorship decreased from the contemporary to the  $+3.5^\circ\text{C}$  treatment, there was no significant difference between those survivorship curves ( $\beta = -1.84 \pm 1.07$ ,  $z = 1.60$ ,  $P = 0.110$ ). Embryonic survival in the field also decreased under warming with 36.9  $\pm$  9.3% survival among natural nests (typical of nest survivorship in SC, Tinkle & Ballinger 1972) versus

**Table 1** Summary statistics for analyses of laboratory and field data using mixed effects ANOVA

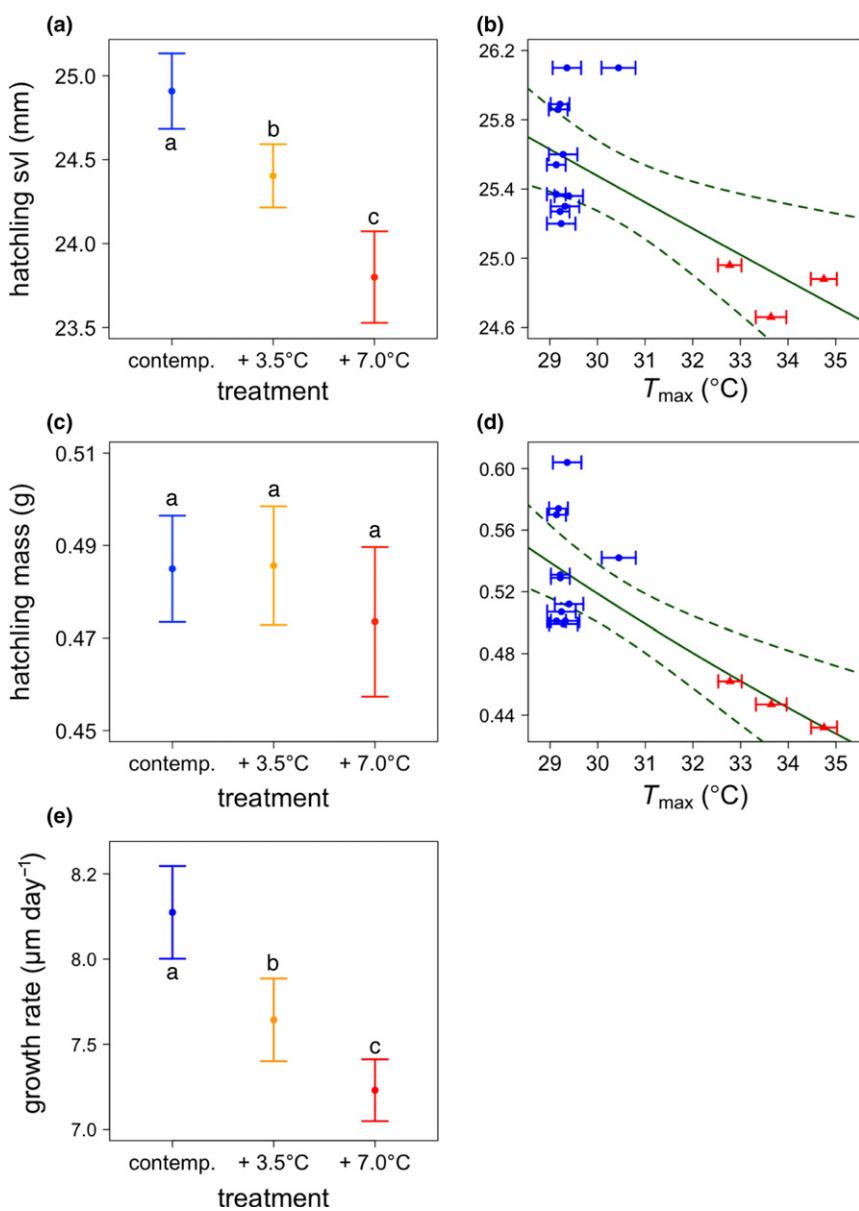
Response	Parameter	F	P	$\omega^2$
<i>Lab Experiment</i>				
(a) Development time	Treatment	<b>108.71</b> <sub>2,63</sub>	< 0.001	<b>0.7521</b>
	Initial egg mass	<b>5.01</b> <sub>1,63</sub>	<b>0.029</b>	0.0140
(b) Hatchling SVL	Treatment	<b>7.16</b> <sub>2,45</sub>	<b>0.002</b>	<b>0.1653</b>
	Initial egg mass	<b>14.22</b> <sub>1,45</sub>	< 0.001	<b>0.1774</b>
(c) Hatchling mass	Treatment	0.32 <sub>2,45</sub>	0.725	0.0000
	Initial egg mass	<b>19.40</b> <sub>1,45</sub>	< 0.001	<b>0.2784</b>
(d) Hatchling SMI	Treatment	0.34 <sub>2,45</sub>	0.713	0.0000
(e) Characteristic growth rate ( $r$ )	Treatment	<b>3876</b> <sub>2,14</sub>	< 0.001	<b>0.3226</b>
	Hatchling SVL	<b>16259</b> <sub>1,14</sub>	< 0.001	<b>0.6769</b>
<i>Field Experiment</i>				
(f) $T_{\max}$	Treatment	<b>438.65</b> <sub>1,792</sub>	< 0.001	<b>0.3553</b>
(g) $T_{\min}$	Treatment	1.35 <sub>1,792</sub>	0.245	0.0004
(h) Embryonic survival	Treatment	<b>14.93</b> <sub>1,6</sub>	<b>0.008</b>	<b>0.6351</b>
(i) Development time	Treatment	<b>12.35</b> <sub>1,12</sub>	<b>0.004</b>	<b>0.4477</b>
(j) Hatchling SVL	Treatment	<b>14.14</b> <sub>1,12</sub>	<b>0.003</b>	<b>0.4842</b>
(k) Hatchling mass	Treatment	<b>16.38</b> <sub>1,12</sub>	<b>0.002</b>	<b>0.5235</b>
(l) Hatchling SMI	Treatment	<b>24.11</b> <sub>1,12</sub>	< 0.001	<b>0.6228</b>

Laboratory data include (a) time to hatching, hatchlings sizes in (b) SVL and (c) mass, (d) hatchling body conditions, and (e) characteristic growth rate derived from the Von Bertalanffy growth models. Laboratory analyses were performed using maternal identity as a random effect. Field data include (f) maximum and (g) minimum daily nest temperatures, (h) embryonic survival, (i) time to hatching, hatchling sizes in (j) SVL and (k) mass, and (l) hatchling body conditions. Analyses of field data included assigned nest and nesting group as a random effect. Bolded values indicate statistical significance.

$\omega^2$ , effect size (Oleinik & Algina 2003); SVL, snout-vent-length; SMI, scaled mass index (Peig & Green 2009, 2010);  $r$ , post-hatching growth rate;  $T_{\max}$ , maximum daily temperature;  $T_{\min}$ , minimum daily temperature.

$7.1 \pm 4.9\%$  among warmed nests (Fig. 1d, Table 1). Lower survivorship in the field than in the laboratory was likely due to differences in hydric conditions. We maintained consistent hydric conditions in the laboratory, whereas embryos in the field experience natural variations in soil moisture that can impact survival (Tracy 1980; Packard *et al.* 1982).

Sublethal warming also led to shorter incubation times and smaller hatchling sizes in both experiments, lower body conditions of hatchlings in the field, and slower post-hatching growth in the laboratory. In the laboratory, hatchlings emerged 12.9% earlier from the  $+3.5^\circ\text{C}$  treatment ( $n = 26$ ,  $-8.93 \pm 0.37$  days) and 15.4% earlier from  $+7.0^\circ\text{C}$  ( $n = 18$ ,  $-10.72 \pm 0.63$  days) compared to the contemporary treatment ( $n = 23$ ,  $69.39 \pm 0.69$  days; Fig. 1e, Table 1). In the field, hatchlings from warmed nests emerged 17.6% earlier ( $n = 3$ ,  $-13.30 \pm 1.20$  days) than from natural nests ( $n = 11$ ,  $75.64 \pm 1.90$  days; Fig. 1f, Table 1). Lizards from laboratory warming treatments hatched at shorter SVL (contemporary:  $n = 17$ ,  $24.91 \pm 0.22$  mm;  $+3.5^\circ\text{C}$ :  $n = 19$ ,  $24.40 \pm 0.19$  mm;  $+7.0^\circ\text{C}$ :  $n = 13$ ,  $23.80 \pm 0.27$  mm; Fig. 2a, Table 1), though hatchling mass and SMI did not differ (contemporary:  $n = 17$ ,  $0.48 \pm 0.01$  g,  $0.486 \pm 0.025$  SMI;  $+3.5^\circ\text{C}$ :  $n = 19$ ,  $0.49 \pm 0.01$  g,  $0.485 \pm 0.023$  SMI;  $+7.0^\circ\text{C}$ :  $n = 13$ ,  $0.47 \pm 0.02$  g,  $0.473 \pm 0.028$  SMI; Fig. 2c, Table 1). In the field, hatchlings emerged from warmed nests at shorter SVL and lighter mass



**Figure 2** Impacts of warming treatments on post-hatching sizes and projected growth rates. Error bars indicate  $\pm 1$  SE. Hatchling snout-vent-length decreased with increased warming (a) in the laboratory and (b) in the field. Hatchling mass decreased with warming nest temperatures (d) in the field, but there was no significant difference in hatchling mass among (c) laboratory treatments. (e) In the laboratory, characteristic growth rates derived from von Bertalanffy growth models decreased with increased warming. For panels a, c, and e, letters denote statistical relationships such that data with different letters are significantly different ( $P < 0.05$ ). See Table 1 for summary statistics.

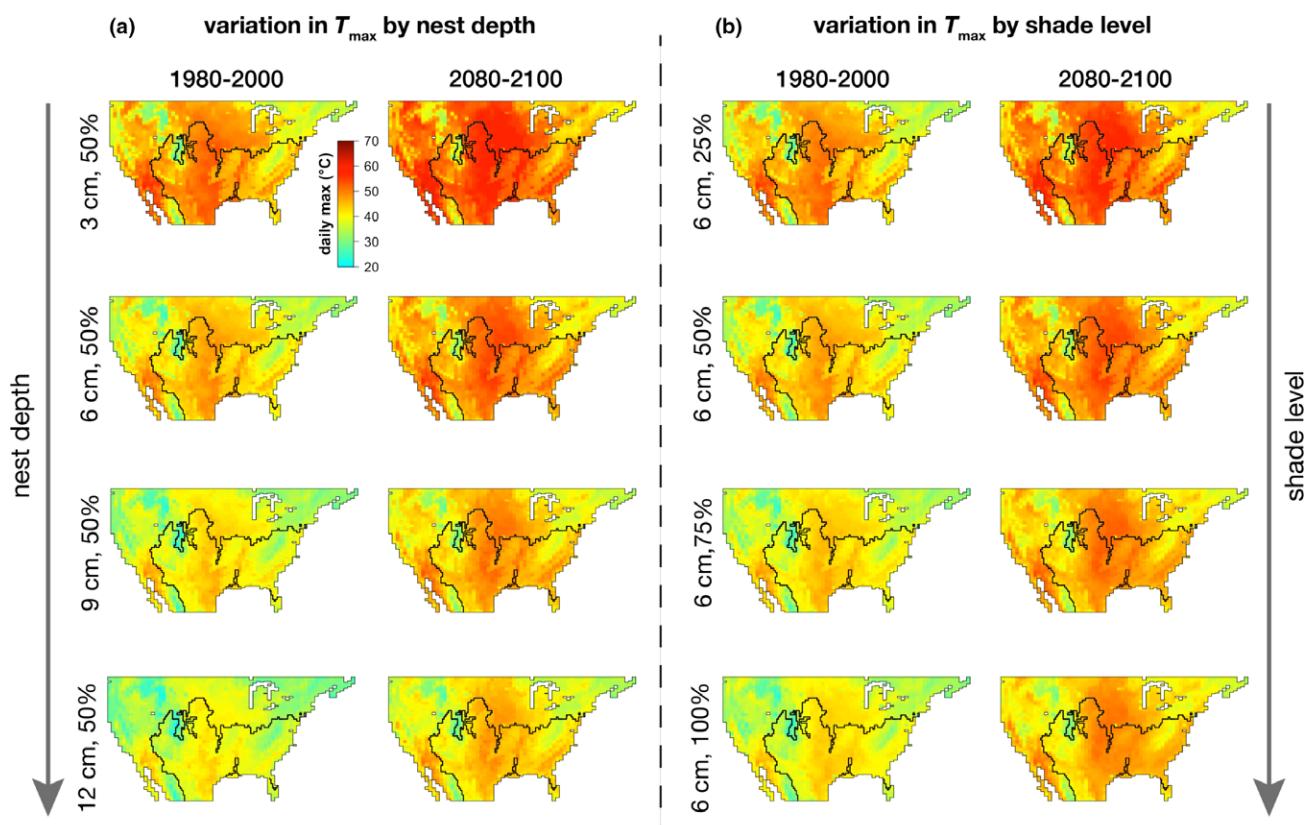
(natural:  $n = 11$ ,  $25.60 \pm 0.10$  mm,  $0.53 \pm 0.01$  g; warmed:  $n = 3$ ,  $24.83 \pm 0.16$  mm,  $0.45 \pm 0.01$  g; Fig. 2b,d, Table 1), which led to lower SMI (natural:  $0.534 \pm 0.019$ , warmed:  $0.447 \pm 0.046$ ; Table 1). The growth model predicted 6.4% lower  $r$  from the  $+3.5^\circ\text{C}$  treatment ( $n = 8$ ,  $7.51 \pm 0.19$   $\mu\text{m day}^{-1}$ ) and 10.5% lower from  $+7.0^\circ\text{C}$  ( $n = 4$ ,  $7.18 \pm 0.14$   $\mu\text{m day}^{-1}$ ) compared to contemporary ( $n = 6$ ,  $8.02 \pm 0.22$   $\mu\text{m day}^{-1}$ ; Fig. 2e, Table 1).

#### Model of population dynamics

Our SDM (herein ‘sublethal model’) predicts more severe consequences of climate warming than those of a model

based solely on lethal limits of embryonic thermotolerances (herein ‘lethal model’). The sublethal model accounts for the fact that nesting conditions avoiding lethal extremes still experience recurrent thermal stressors (Fig. 3; Figs S1–S14). By accounting for moderate warming, we demonstrate that even small changes in temperature can lead to increased risk of extirpation under contemporary and future climates.

Predicted embryonic survival decreases under contemporary and future climates when incorporating our empirical observations. Under typical nesting conditions in July (6 cm-depth and 50%-shade, Angilletta *et al.* 2009; 4.4–8.0 cm and 51.6–63.5%, this manuscript), the sublethal model predicts lower



**Figure 3** Spatial distributions of average maximum daily temperatures ( $T_{\max}$ ) during the month of July for the period 1980–2000 and predicted for the period 2080–2100. Black outlines within maps indicate the extant *Sceloporus undulatus* range (IUCN 2017). Variation in  $T_{\max}$  is displayed across (a) increasing nest depths under 50% shade and (b) across increasing shade levels at 6 cm nest depth. See Figs S1–S14 for plots based on all other combinations of nest depth (3, 6, 9, or 12 cm) and shade (0, 25, 50, 75, or 100%) and for nests laid in April, May, June, August, September and October.

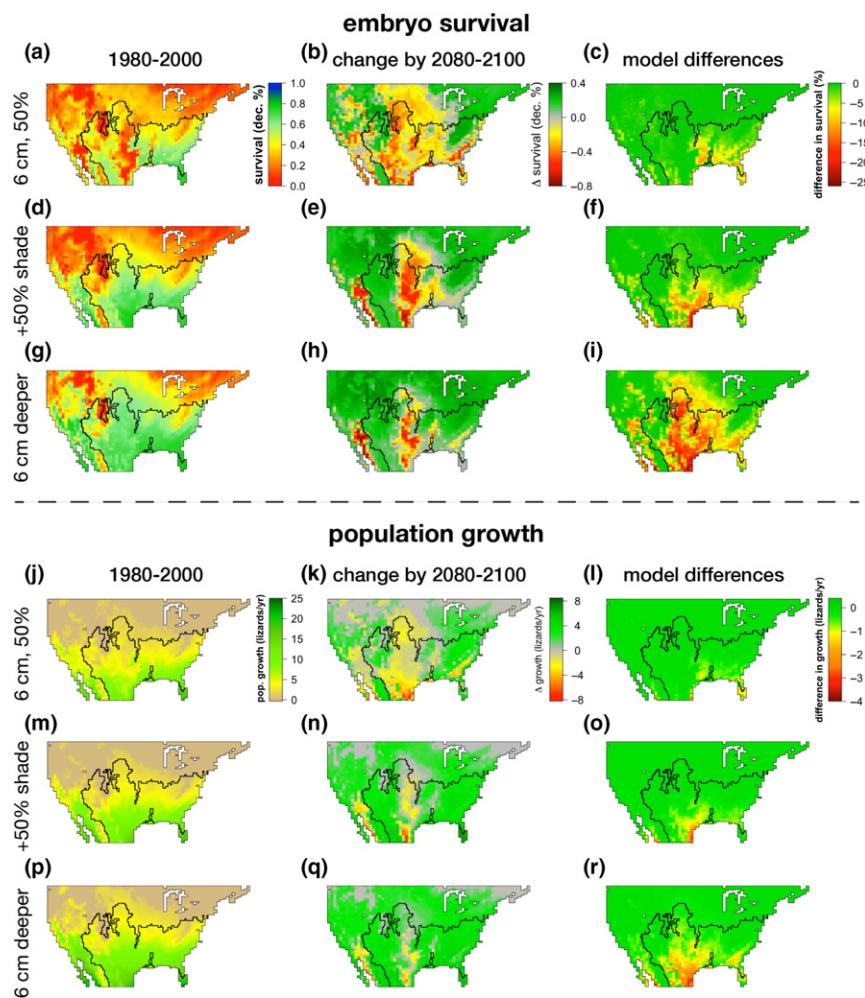
survival across 82.6% of the species range by  $-2.2\%$  on average and by as much as  $-12.0\%$  in locales that experience lower temperature variance, including portions of the southeast, the central plains, and the southwest (Fig. 4c). The magnitude and distribution of differences in predicted survival varies with nest depth, shade, and timing of oviposition (Figs 4a–i and 5, Figs S15–S42). For instance, incorporating the effects of sublethal warming alters survival across 96.8% of the range by  $-7.8\%$  on average and by as much as  $-23.8\%$  for nests laid in July at 12 cm depth and 50% shade (Fig. 4i). Reduced embryonic survival then leads to decreased projected population growth.

Recurrent sublethal warming during incubation leads to decreased projected population growth. Both models show positive population growth across 96.0% of the species range under contemporary nesting conditions. Yet, when accounting for sublethal warming, the majority (84.7%) of those areas with positive growth experience increased risk of extirpation due to reduced population growth rates. Both models also agree on the geographic area of decreases in population growth under future warming (e.g., 51.4% and 50.5% of the range from the lethal and sublethal models, respectively, for typical nesting conditions). However, the magnitudes of reduced growth differ between the models. By overestimating embryonic survival, the lethal model underestimates negative impacts on population growth across 92.7% of the species

range by 3.2% on average and by as much as 12.2% in locales that experience lower temperature variance (Fig. 4). Differences in population growth projections vary with nest depth, shade, timing and geography similarly to embryonic survival (Fig. 4j–r, Figs S43–S46).

Sensitivity analyses examined how changes in hatchling sizes and juvenile growth rates affected projections of population growth via changes time to maturity. The growth model indicated increased age at maturity by  $32.4 \pm 7.6$  days across the species range when incorporating slowed juvenile growth (Fig. S48). In SC, a predicted 26-day delay in maturity could reduce population growth rates up to an additional 39.7% over the 24.4% predicted by the sublethal model. In NJ, population growth rates could decrease by an additional 80.1% due to a 29-day delay in maturity, which would lead to population decline and likely extirpation. These results demonstrate potentially severe impacts of sublethal warming during incubation on population dynamics via downstream effects through ontogeny.

After comparing projections, we evaluated how well predictions match the contemporary species distribution. Both models predict the contemporary extent of the species range equally well if we treat positive embryonic survival and population growth as the only criteria. We also calculated sensitivity indices (proportion of presences predicted with positive survival, Manel *et al.* 2001; Buckley *et al.* 2010) and found no



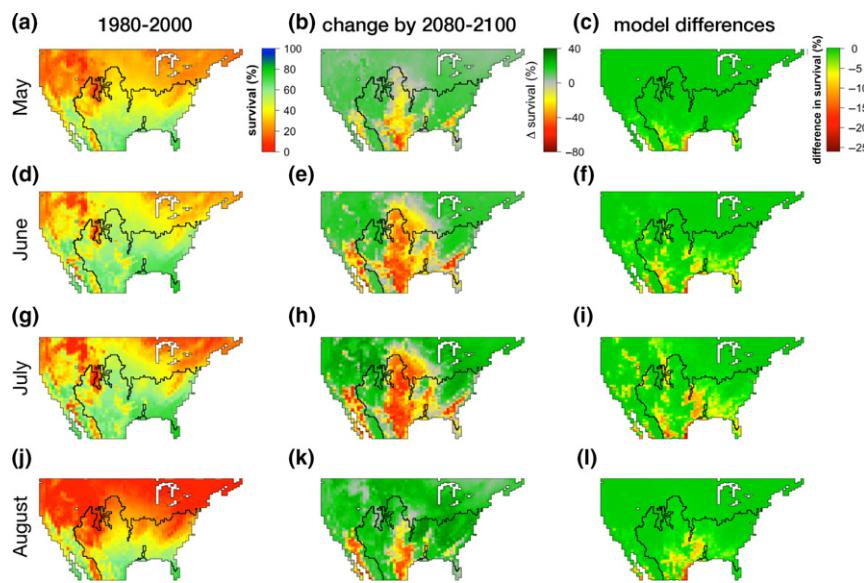
**Figure 4** Spatial distributions of embryonic survival and population growth rates generated by the sublethal model for the period 1980–2000, changes by 2080–2100, and differences between these projections and those generated by the lethal model. Negative model differences indicate the degree to which predictions are reduced by incorporating effects of moderate warming. Black outlines within maps indicate the extant *Sceloporus undulatus* range (IUCN 2017). Results are shown at three scenarios of nesting behaviour: (a–c, j–l) 6 cm depth and 50% shade typical of *S. undulatus* (Angilletta *et al.* 2009; this manuscript), (d–f, m–o) nest sites with 50% more shade, and (g–i, p–r) nests dug 6 cm deeper. Survival results are based on simulations for nests laid in July. See Figs S15–S42 for survival plots at all other combinations of nest depth (3, 6, 9, or 12 cm) and shade (0, 25, 50, 75, or 100%) and for nests laid in April, May, June, August, September, and October. Also, see Figs S43–S46 for population growth plots based on all other combinations of nest depth and shade.

differences (see Appendix S1 for details). However, embryonic survival under the sublethal model decreased across 74.4% of occurrences to rates more consistent with demographic data (Tinkle & Ballinger 1972; Vinegar 1975; Tinkle & Dunham 1986). Thus, consideration of fluctuating developmental conditions reveals vulnerability to climate change that is not apparent without examination of sublethal warming.

## DISCUSSION

We have demonstrated that organisms with thermally sensitive life stages do not have to experience lethal temperatures to undergo negative changes at the individual and population levels. Explicitly testing the effects of increasing  $T_{\max}$  showed decreased embryonic survival under recurrent sublethal warming. The effects of warming extended through later life stages via reduced body condition and slowed growth. By integrating survivorship results into an SDM, we show that consideration

of moderate warming during vulnerable life stages alters predicted impacts of climate change. Shifts in distributions result from both lethal conditions (Jones *et al.* 2010; WetHEY *et al.* 2011; Levy *et al.* 2015) and chronic exposure to sublethal fluctuations (Fly & Hilbish 2013; Woodin *et al.* 2013; Maynard *et al.* 2015). Numerous studies demonstrate that changing mean incubation temperatures affect phenotypes of oviparous ectotherms (e.g., reviews in Deeming & Ferguson 1991a; Booth 2006; Bowden *et al.* 2014), and variance of incubation temperatures affects traits across ontogeny as strongly or more than increasing means (e.g., Shine & Harlow 1996; Paaijmans *et al.* 2013). In the *Sceloporus* system, warming of constant and fluctuating incubation regimes can speed development without impacting hatchling sizes (review in Angilletta *et al.* 2004b). However, studies using fluctuating temperatures did not reach stressful highs (except Levy *et al.* 2015; but see below). In this study, survival decreased as the mean and variance of embryonic temperatures increased



**Figure 5** Spatial distributions of predicted embryonic survival generated by the sublethal model for the period 1980–2000, predicted changes by 2080–2100, and differences between these projections and those generated by the lethal model. Negative model differences indicate the degree to which predictions are reduced by incorporating effects of moderate warming. Black outlines within maps indicate the extant *Sceloporus undulatus* range (IUCN 2017). Results are shown across months in the breeding season to illustrate differences based on the timing of oviposition. These results are based on simulations for nests laid at 9 cm depth and 50% shade. See Figs S15–S42 for survival plots based on all other combinations of nest depth (3, 6, 9, or 12 cm) and shade (0, 25, 50, 75, or 100%) and for nests laid in April, September, and October.

beyond that experienced in contemporary nests. We cannot partition the effects of temperature means and variances in our experiments. Yet, biological impacts of climate warming likely result from interactions between thermal means and variances, which are presumably not independent of one another in natural microclimates (Shine & Harlow 1996; Paaijmans *et al.* 2013; Bozinovic *et al.* 2015). By utilising naturalistic thermal regimes, we demonstrate how impacts of warming on sensitive periods of ontogeny can affect ecological predictions.

Our SDM indicates that moderate warming during incubation can lead to reduced population growth compared to model predictions that do not incorporate sublethal fluctuations. Interestingly, the differences in laboratory survivorship that altered model predictions stemmed primarily from mortality in the first weeks post-oviposition. Running the survival analysis for the first 25% of the incubation period showed lower survival probability under the +7.0°C treatment before any mortality events in the other treatments. Levy *et al.* (2015) suggested similar levels of warming had no effect on *S. undulatus* embryo survival (Levy *et al.* 2015), but they did not begin treatments until halfway through incubation. Our results suggest increased sensitivity to thermal stress in the earliest stages post-oviposition, during which incidences of developmental abnormalities increase as incubation temperatures near the lethal limits for reptiles and other ectotherms (reviews in Deeming & Ferguson 1991b; Farmer 2000). Therefore, *in situ* examinations of plasticity in nesting behaviour could be critical to predicting the susceptibility of many ectotherms to climate change.

Plasticity of maternal behaviour could buffer embryos from negative effects of climate change (Telemeco *et al.* 2009; Levy

*et al.* 2015). However, the benefit of compensatory nesting behaviour diminishes when accounting for effects of sublethal warming. Our model examines scenarios of altered nesting behaviour by simulating oviposition across ranges of nest depths, shades, and days of the year beyond that exhibited among contemporary *S. undulatus* populations (Tinkle & Ballinger 1972; Niewiarowski 1994; Angilletta *et al.* 2009; this manuscript). Per the sublethal model, embryonic survival will decrease across much of the species range regardless of phenology (Fig. 5; though see Levy *et al.* 2016b). Nests with lower temperature variance could reduce negative impacts of warming by avoiding lethal extremes, but the impacts of sublethal warming may constrain that mitigation. For instance, if females nest 3 cm deeper than contemporary averages, the sublethal model predicts a 17.4% lower increase in embryonic survival at the end of this century than the 179.2% benefit predicted by the lethal model. Repeated exposure to sublethal highs can be more detrimental to fitness than acute exposure to extreme temperatures for some species (Kearney *et al.* 2012; Marshall & Sinclair 2015). Thus, the effects of sublethal warming drive responses to warming through impacts on development and stage-specific mortality.

We demonstrate that warming during incubation could have significant impacts on demography via stage-specific survival and growth. Recurrent sublethal warming decreased embryo survival. Additionally, it led to smaller hatchlings and slowed juvenile growth, which could decrease survival to maturity via increased predation risk and reduced foraging success (Sinervo 1993; Stearns 2000; Sears & Angilletta 2004). One could argue that a longer growing season under warming mean temperatures could compensate for slowed juvenile growth. However, increased temperature variance would likely counteract such

benefits via constrained activity time and more frequent potential for heat stress (Kingsolver *et al.* 2013; Levy *et al.* 2016b). Additionally, epigenetic effects could compensate for negative impacts of incubation conditions, such that exposure to warming during early ontogeny increases survival and performance of later stages. Though that is beyond the scope of this study, we incorporated predictions of embryonic survival and time to maturity into life tables to examine how slowed juvenile growth could negatively impact population persistence. Though assumptions in our model preclude life-history variation across geography, our life tables include such differences and highlight potentially severe downstream consequences of recurrent sublethal warming during incubation; results indicate particularly strong effects in northern populations that already exhibit delayed maturity compared to southern populations (Tinkle & Ballinger 1972; Niewiarowski 1994). Future integration of geographic variation of life-history traits will further improve model predictions.

According to life-history theory, faster growth should occur in environments where juveniles experience low survivorship (Stearns 2000), and *S. undulatus* juveniles grow more quickly and experience higher mortality at more southern latitudes (Angilletta *et al.* 2004a; Sears & Angilletta 2004). Our novel nest temperature data demonstrate a counterintuitive pattern wherein southern embryos experience cooler temperatures than their northern conspecifics (Angilletta *et al.* 2009). Considering our results, one could hypothesise that variation in nest characteristics may be a mechanism underlying geographic variation in life-history traits in this species. Further research, such as reciprocal transplants of *S. undulatus* embryos across latitudes, could address hypotheses concerning plasticity of life-history traits (e.g., Stearns & Koella 1986) and elucidate impacts of nesting behaviour and embryo thermal physiology on such variation. Accordingly, our work demonstrates the need for increased focus on ontogenetic and spatiotemporal variation of organismal responses to environmental fluctuations.

Our results should motivate researchers to expand efforts to examine life-cycle responses to local climates. If moderate warming during development can impede recruitment and decrease mean fitness, species in locations with lower thermal variance and relatively low frequencies of extreme events may suffer more than previously thought under climate warming. Unfortunately, data on responses to sublethal extremes are not sufficiently available to inform models beyond a few well-studied systems, such as corals (e.g., Edmunds 2005; Maynard *et al.* 2015), intertidal mussels (e.g., Miller *et al.* 2009; Fly & Hilbush 2013), and some insect species (e.g., Crozier & Dwyer 2006; Potter *et al.* 2011; Marshall & Sinclair 2015). The enduring impacts of sublethal environmental fluctuations is a largely unaddressed problem in ecological modelling. Future studies should examine responses to spatiotemporal variation in developmental conditions to further elucidate adaptive processes by which organisms handle environmental fluctuations.

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## AUTHORSHIP

MAC and MWS designed the laboratory and field studies, with consultation from EAR. OL designed the species distribution model. MAC collected data and analysed model output. MAC wrote the first draft, and all authors contributed to revisions.

## DATA ACCESSIBILITY STATEMENT

Data available from the Dryad Digital Repository: <http://doi.org/10.5061/dryad.pr1h0>.

## CONFLICT OF INTEREST

The authors declare no competing financial interests.

## REFERENCES

- Andrews, R.M., Qualls, C.P. & Rose, B.R. (1997). Effects of low temperature on embryonic development of *Sceloporus* lizards. *Copeia*, 1997, 827–833.
- Andrews, R.M., Mathies, T. & Warner, D.A. (2000). Effect of incubation temperature on morphology, growth, and survival of juvenile *Sceloporus undulatus*. *Herpetol. Monogr.*, 14, 420–431.
- Angilletta, M.J. Jr (2001). Thermal and physiological constraints on energy assimilation in a widespread lizard (*Sceloporus undulatus*). *Ecology*, 82, 3044–3056.
- Angilletta, M.J. Jr, Winters, R.S. & Dunham, A.E. (2000). Thermal effects on the energetics of lizard embryos: implications for hatchling phenotypes. *Ecology*, 81, 2957–2968.
- Angilletta, M.J. Jr, Oufiero, C.E. & Sears, M.W. (2004a). Thermal adaptation of maternal and embryonic phenotypes in a geographically widespread ectotherm. *Int. Congr. Ser.*, 1275, 258–266.
- Angilletta, M.J., Steury, T.D. & Sears, M.W. (2004b). Temperature, growth rate, and body size in ectotherms: fitting pieces of a life-history puzzle. *Integr. Comp. Biol.*, 44, 498–509.
- Angilletta, M.J., Sears, M.W. & Pringle, R.M. (2009). Spatial dynamics of nesting behaviour: lizards shift microhabitats to construct nests with beneficial thermal properties. *Ecology*, 90, 2933–2939.
- Angilletta, M.J., Zelic, M.H. & Adrian, G.J. (2013). Heat tolerance during embryonic development has not diverged among populations of a widespread species (*Sceloporus undulatus*). *Conserv. Physiol.*, 1, 1–9.
- Badyaev, A.V. (2005). Stress-induced variation in evolution: from behavioural plasticity to genetic assimilation. *Proc. R. Soc. Lond. B*, 272, 877–886.
- Bakken, G.S. (1992). Measurement and application of operative and standard operative temperatures in ecology. *Am. Zool.*, 32, 194–216.
- Booth, D.T. (2006). Influence of incubation temperature on hatchling phenotype in reptiles. *Physiol. Biochem. Zool.*, 79, 274–281.

- Bowden, R.M., Carter, A.W. & Paitz, R.T. (2014). Constancy in an inconstant world: moving beyond constant temperatures in the study of reptilian incubation. *Integr. Comp. Biol.*, 54, 830–840.
- Bozinovic, F., Bastías, D.A., Boher, F., Clavijo-Baquet, S., Estay, S.A. & Angilletta, M.J. Jr (2015). The mean and variance of environmental temperature interact to determine physiological tolerance and fitness. *Physiol. Biochem. Zool.*, 84, 543–552.
- Buckley, L.B. (2008). Linking traits to energetics and population dynamics to predict lizard ranges in changing environments. *Am. Nat.*, 171, E1–E19.
- Buckley, L.B., Urban, M.C., Angilletta, M.J., Crozier, L.G., Rissler, L.J. & Sears, M.W. (2010). Can mechanism inform species' distribution models? *Ecol. Lett.*, 13, 1041–1054.
- Campbell, W.B., Emlen, J.M. & Hershberger, W.K. (1998). Thermally induced chronic developmental stress in coho salmon: integrating measures of mortality, early growth, and developmental instability. *Oikos*, 81, 398–410.
- Castro, J., Zamora, R., Hódar, J.A. & Gómez, J.M. (2005). Alleviation of summer drought boosts establishment success of *Pinus sylvestris* in a Mediterranean mountain: an experimental approach. *Plant Ecol.*, 181, 191–202.
- Christian, K.A., Tracy, C.R. & Porter, W.P. (1986). The effect of cold exposure during incubation of *Sceloporus undulatus* eggs. *Copeia*, 1986, 1012–1014.
- Crozier, L. & Dwyer, G. (2006). Combining population-dynamic and ecophysiological models to predict climate-induced insect range shifts. *Am. Nat.*, 167, 853–866.
- Crozier, L.G., Zabel, R.W. & Hamlet, A.F. (2008). Predicting differential effects of climate change at the population level with life-cycle models of spring Chinook salmon. *Glob. Change Biol.*, 14, 236–249.
- De Block, M. & Stoks, R. (2005). Fitness effects from egg to reproduction: bridging the life history transition. *Ecology*, 86, 185–197.
- Deeming, D.C. & Ferguson, M.W.J. (1991a). *Egg Incubation: Its Effects on Embryonic Development in Birds and Reptiles*. Cambridge University Press, Cambridge, UK.
- Deeming, D.C. & Ferguson, M.W.J. (1991b). Physiological effects of incubation temperature on embryonic development in reptiles and birds. In *Egg Incubation: Its Effects on Embryonic Development in Birds and Reptiles* (eds Deeming, D.C., Ferguson, M.W.J.). Cambridge University Press, Cambridge, UK, pp. 147–171.
- Deutsch, C.A., Tewksbury, J.J., Huey, R.B., Sheldon, K.S., Ghalambor, C.K., Haak, D.C., et al. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proc. Natl Acad. Sci. USA*, 105, 6668–6672.
- Donat, M.G. & Alexander, L.V. (2012). The shifting probability distribution of global daytime and night-time temperatures. *Geophys. Res. Lett.*, 39, L14707.
- Du, W.G. & Shine, R. (2015). The behavioural and physiological strategies of bird and reptile embryos in response to unpredictable variation in nest temperature. *Biol. Rev.*, 90, 19–30.
- Dunham, A.E. (1978). Food availability as a proximate factor influencing individual growth rates in the iguanid lizard *Sceloporus merriami*. *Ecology*, 59, 770–778.
- DuRant, S.E., Hepp, G.R., Moore, I.T., Hopkins, B.C. & Hopkins, W.A. (2010). Slight differences in incubation temperature affect early growth and stress endocrinology of wood duck (*Aix sponsa*) ducklings. *J. Exp. Biol.*, 213, 45–51.
- Edmunds, P.J. (2005). The effect of sub-lethal increases in temperature on the growth and population trajectories of three scleractinian corals on the southern Great Barrier Reef. *Oecologia*, 146, 350–364.
- Elzhov, T.V., Mullen, K.M., Spiess, A.-N. & Bolker, B. (2015). minpack.lm: R Interface to the Levenberg-Marquardt nonlinear least-squares algorithm found in MINPACK, plus support for bounds. R package version 1.1-9. Available at: <http://CRAN.R-project.org/package=minpack.lm>.
- Farmer, C.G. (2000). Parental care: the key to understanding endothermy and other convergent features in birds and mammals. *Am. Nat.*, 155, 326–334.
- Fly, E.K. & Hilbish, T.J. (2013). Physiological energetics and biogeographic range limits of three congeneric mussel species. *Oecologia*, 172, 35–46.
- Georges, A., Beggs, K., Young, J.E. & Doody, J.S. (2005). Modelling development of reptile embryos under fluctuating temperature regimes. *Physiol. Biochem. Zool.*, 78, 18–30.
- Harrison, X.A., Blount, J.D., Inger, R., Norris, D.R. & Bearhop, S. (2011). Carry-over effects as drivers of fitness differences in animals. *J. Anim. Ecol.*, 80, 4–18.
- Haywood, S. & Perrins, C.M. (1992). Is clutch size in birds affected by environmental conditions during growth? *Proc. R. Soc. Lond. B*, 249, 195–197.
- Helmhuth, B., Kingsolver, J.G. & Carrington, E. (2005). Biophysics, physiological ecology, and climate change: does mechanism matter? *Annu. Rev. Physiol.*, 67, 177–201.
- Hepp, G.R., Kennamer, R.A. & Johnson, M.H. (2006). Maternal effects in wood ducks: incubation temperature influences incubation period and neonate phenotype. *Funct. Ecol.*, 20, 307–314.
- IPCC (2013). Climate Change 2013: the physical science basis. In: *Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental panel on Climate Change* (eds Stocker, T.F., Qin, D., Plattner, G.K., Tignor, M., Allen, S.K. & Boschung, J.). Cambridge University Press, Cambridge, NY, USA, 1535 pp.
- IUCN (2017). The IUCN red list of threatened species. Version 2017-1. Available at: <http://www.iucnredlist.org>. Last accessed 27 September 2016.
- Jones, S.J., Lima, F.P. & Wethey, D.S. (2010). Rising environmental temperatures and biogeography: poleward range contraction of the blue mussel, *Mytilus edulis* L., in the western Atlantic. *J. Biogeogr.*, 37, 2243–2259.
- Kearney, M.R. (2013). Activity restriction and the mechanistic basis for extinctions under climate warming. *Ecol. Lett.*, 16, 1470–1479.
- Kearney, M.R., Matzelle, A. & Helmuth, B. (2012). Biomechanics meets the ecological niche: the importance of temporal data resolution. *J. Exp. Biol.*, 215, 1422–1424.
- Kingsolver, J.G., Diamond, S.E. & Buckley, L.B. (2013). Heat stress and the fitness consequences of climate change for terrestrial ectotherms. *Funct. Ecol.*, 27, 1415–1423.
- Kingsolver, J.G., Higgins, J.K. & Augustine, K.E. (2015). Fluctuating temperatures and ectotherm growth: distinguishing nonlinear and time-dependent effects. *J. Exp. Biol.*, 218, 2218–2225.
- Kingsolver, J.G., Woods, H.A., Buckley, L.B., Potter, K.A., MacLean, H.J. & Higgins, J.K. (2011). Complex life cycles and the responses of insects to climate change. *Integr Comp Biol*, 51, 719–732.
- Larios, E., Bürquez, A., Becerra, J.X. & Lawrence Venable, D. (2014). Natural selection on seed size through the life cycle of a desert annual plant. *Ecology*, 95, 3213–3220.
- Levy, O., Buckley, L.B., Keitt, T.H., Smith, C.D., Boateng, K.O., Kumar, D.S., et al. (2015). Resolving the life cycle alters expected impacts of climate change. *Proc. R. Soc. Lond. B*, 282, 20150837.
- Levy, O., Buckley, L.B., Keitt, T.H. & Angilletta, M.J. (2016a). A dynamically downscaled projection of past and future microclimates. *Ecology*, 97, 1888.
- Levy, O., Buckley, L.B., Keitt, T.H. & Angilletta, M.J. (2016b). Ontogeny constrains phenology: opportunities for activity and reproduction interact to dictate potential phenologies in a changing climate. *Ecol. Lett.*, 19, 620–628.
- Lindström, J. (1999). Early development and fitness in birds and mammals. *Trends Ecol. Evol.*, 14, 343–348.
- Lumey, L.H. & Stein, A.D. (1997). In utero exposure to famine and subsequent fertility: the Dutch famine birth cohort study. *Am. J. Public Health*, 87, 1962–1966.
- Manel, S., Williams, H.C. & Ormerod, S.J. (2001). Evaluating presence-absence models in ecology: the need to account for prevalence. *J. Appl. Ecol.*, 38, 921–931.
- Marshall, K.E. & Sinclair, B.J. (2015). The relative importance of number, duration and intensity of cold stress events in determining

- survival and energetics of an overwintering insect. *Funct. Ecol.*, 29, 357–366.
- Maynard, J., van Hoodonk, R., Eakin, C.M., Puotinen, M., Garren, M., Williams, G., et al. (2015). Projections of climate conditions that increase coral disease susceptibility and pathogen abundance and virulence. *Nat. Clim. Chang.*, 5, 688–694.
- Meehl, G.A. & Tebaldi, C. (2004). More intense, more frequent, and longer lasting heat waves in the 21st century. *Science*, 305, 994–997.
- Miller, L.P., Harley, C. & Denny, M.W. (2009). The role of temperature and desiccation stress in limiting the local-scale distribution of the owl limpet, *Lottia gigantea*. *Funct. Ecol.*, 23, 756–767.
- Neilson, R.P., Pitelka, L.F., Solomon, A.M., Nathan, R., Midgley, G.F., Fragoso, J.M.V., et al. (2005). Forecasting regional to global plant migration in response to climate change. *Bioscience*, 55, 749–759.
- Niehaus, A.C., Angilletta, M.J., Sears, M.W., Franklin, C.E. & Wilson, R.S. (2012). Predicting the physiological performance of ectotherms in fluctuating thermal environments. *J. Exp. Biol.*, 215, 694–701.
- Niewiarowski, P.H. (1992). Ecological and evolutionary sources of geographic variation in individual growth rates of the lizard *Sceloporus undulatus* (Iguanidae). PhD Dissertation, University of Pennsylvania.
- Niewiarowski, P.H. (1994). Understanding geographic life-history variation in lizards. In *Lizard Ecology: Historical and Experimental Perspectives* (eds Pianka, E.R., Vitt, L.J.). Princeton University Press, Princeton, NJ, USA, pp. 31–50.
- Olejnik, S. & Algina, J. (2003). Generalized eta and omega squared statistics: measures of effect size for some common research designs. *Psychol. Methods*, 8, 434–447.
- Oufiero, C.E. & Angilletta, M.J. (2006). Convergent evolution of embryonic growth and development in the eastern fence lizard (*Sceloporus undulatus*). *Evolution*, 60, 1066–1075.
- Overall, K.L. (1994). Lizard egg environments. In *Lizard Ecology: Historical and Experimental Perspectives* (eds Pianka, E.R., Vitt, L.J.). Princeton University Press, Princeton, NJ, USA, pp. 51–72.
- Paaijmans, K.P., Heinig, R.L., Seliga, R.A., Blanford, J.I., Blanford, S., Murdock, C.C., et al. (2013). Temperature variation makes ectotherms more sensitive to climate change. *Glob. Change Biol.*, 19, 2373–2380.
- Pacifici, M., Foden, W.B., Visconti, P. & Watson, J. (2015). Assessing species vulnerability to climate change. *Nature*, 5, 215–225.
- Packard, M.J., Packard, G.C. & Boardman, T.J. (1982). Structure of eggshells and water relations of reptilian eggs. *Herpetologica*, 38, 136–155.
- Parker, S.L., Andrews, R.M. & Mathies, T. (2004). Embryonic responses to variation in oviductal oxygen in the lizard *Sceloporus undulatus* from New Jersey and South Carolina, USA. *Biol. J. Linn. Soc. Lond.*, 83, 289–299.
- Peig, J. & Green, A.J. (2009). New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. *Oikos*, 118, 1883–1891.
- Peig, J. & Green, A.J. (2010). The paradigm of body condition: a critical reappraisal of current methods based on mass and length. *Funct. Ecol.*, 24, 1323–1332.
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. & R Core Team (2016). nlme: Linear and Nonlinear Mixed Effects Models\_. R package version 3.1-128. Available at: <http://CRAN.R-project.org/package=nlme>.
- Podolsky, R.D. & Moran, A.L. (2006). Integrating function across marine life cycles. *Integr. Comp. Biol.*, 46, 577–586.
- Potter, K.A., Davidowitz, G. & Woods, H.A. (2011). Cross-stage consequences of egg temperature in the insect *Manduca sexta*. *Funct. Ecol.*, 25, 538–556.
- R Core Team (2016). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. Available at: <http://www.R-project.org/>.
- Radchuk, V., Turlure, C. & Schtickzelle, N. (2013). Each life stage matters: the importance of assessing the response to climate change over the complete life cycle in butterflies. *J. Anim. Ecol.*, 82, 275–285.
- Randin, C.F., Engler, R. & Normand, S. (2009). Climate change and plant distribution: local models predict high-elevation persistence. *Glob. Change Biol.*, 15, 1557–1569.
- Refsnider, J.M. & Janzen, F.J. (2010). Putting eggs in one basket: ecological and evolutionary hypotheses for variation in oviposition-site choice. *Annu. Rev. Ecol. Evol. Syst.*, 41, 39–57.
- Riddell, E.A., Apanovitch, E.K., Odom, J.P. & Sears, M.W. (2017). Physical calculations of resistance to water loss improve predictions of species range models. *Ecol. Monogr.*, 87, 21–33.
- Schoener, T.W. & Schoener, A. (1978). Estimating and interpreting body-size growth in some *Anolis* lizards. *Copeia*, 1978, 390–405.
- Sears, M.W. (2005). Geographic variation in the life history of the sagebrush lizard: the role of thermal constraints on activity. *Oecologia*, 143, 25–36.
- Sears, M.W. & Angilletta, M.J. (2004). Body size clines in sceloporus lizards: proximate mechanisms and demographic constraints. *Integr. Comp. Biol.*, 44, 433–442.
- Sexton, O.J. & Marion, K.R. (1974). Duration of incubation of *Sceloporus undulatus* eggs at constant temperature. *Physiol. Zool.*, 47, 91–98.
- Shine, R. & Elphick, M.J. (2001). The effect of short-term weather fluctuations on temperatures inside lizard nests, and on the phenotypic traits of hatchling lizards. *Biol. J. Linn. Soc.*, 72, 555–565.
- Shine, R. & Harlow, P.S. (1996). Maternal manipulation of offspring phenotypes via nest-site selection in an oviparous lizard. *Ecology*, 77, 1808–1817.
- Sinervo, B. (1993). The effect of offspring size on physiology and life history. *Bioscience*, 43, 210–218.
- Stearns, S.C. (2000). Life history evolution: successes, limitations, and prospects. *Naturwissenschaften*, 87, 476–486.
- Stearns, S.C. & Koella, J.C. (1986). The evolution of phenotypic plasticity in life-history traits: predictions of reaction norms for age and size at maturity. *Evolution*, 40, 893–913.
- Sykes, M.T., Prentice, I.C. & Cramer, W. (1996). A bioclimatic model for the potential distributions of north European tree species under present and future climates. *J. Biogeogr.*, 23, 203–233.
- Telemeco, R.S., Elphick, M.J. & Shine, R. (2009). Nesting lizards (*Bassiana duperreyi*) compensate partly, but not completely, for climate change. *Ecology*, 90, 17–22.
- Telemeco, R.S., Gangloff, E.J., Cordero, G.A., Mitchell, T.S., Bodensteiner, B.L., Holden, K.G., et al. (2016). Reptile embryos lack the opportunity to thermoregulate by moving within the egg. *Am. Nat.*, 188, E13–E27.
- Therneau, T.M. (2014). A package for survival analysis in S. R package version 2.37-7. Available at: <http://CRAN.R-project.org/package=survival>.
- Tinkle, D.W. (1969). The concept of reproductive effort and its relation to the evolution of life histories of lizards. *Am. Nat.*, 103, 501–516.
- Tinkle, D.W. & Ballinger, R.E. (1972). *Sceloporus undulatus*: a study of the intraspecific comparative demography of a lizard. *Ecology*, 53, 570–584.
- Tinkle, D.W. & Dunham, A.E. (1986). Comparative life histories of two syntopic sceloporine lizards. *Copeia*, 1986, 1–18.
- Tracy, C.R. (1980). Water Relations of Parchment-Shelled Lizard (*Sceloporus undulatus*) Eggs. *Copeia*, 1980, 478.
- Urban, M.C., Bocedi, G., Hendry, A.P., Mihoub, J.B., Peer, G., Singer, A., et al. (2016). Improving the forecast for biodiversity under climate change. *Science*, 353, aad8466.
- Vinegar, M.B. (1975). Life history phenomena in two populations of the lizard *Sceloporus undulatus* in southwestern New Mexico. *Am. Mid. Nat.*, 93, 388–402.
- Warner, D.A. (2014). Fitness consequences of maternal and embryonic responses to environmental variation: using reptiles as models for studies of developmental plasticity. *Integr. Comp. Biol.*, 54, 757–773.
- Warner, D.A. & Andrews, R.M. (2002). Laboratory and field experiments identify sources of variation in phenotypes and survival of hatchling lizards. *Biol. J. Linn. Soc.*, 76, 105–124.
- WetHEY, D.S., Brin, L.D., Helmuth, B. & Mislan, K. (2011). Predicting intertidal organism temperatures with modified land surface models. *Ecol. Model.*, 222, 3568–3576.

- Woodin, S.A., Hilbish, T.J., Helmuth, B., Jones, S.J. & Wethey, D.S. (2013). Climate change, species distribution models, and physiological performance metrics: predicting when biogeographic models are likely to fail. *Ecol. Evol.*, 3, 3334–3346.
- Wu, T.H., Shiao, S.F. & Okuyama, T. (2015). Development of insects under fluctuating temperature: a review and case study. *J. Appl. Entomol.*, 139, 592–599.
- Zalom, F.G., Goodell, P., Wilson, L.T. & Bentley, W.J. (1983). *Degree-Days: The Calculation and Use of Heat Units in Pest Management*. University of California Division of Agriculture and Natural Resources Leaflet 21373. Division of Agriculture and Natural Resources, Davis, California.

## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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