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RESEARCH ARTICLE

Thermal spikes from the urban heat island increase mortality and alter physiology of lizard embryos

Joshua M. Hall* and Daniel A. Warner

ABSTRACT

Effects of global change (i.e. urbanization, climate change) on adult organisms are readily used to predict the persistence of populations. However, effects on embryo survival and patterns of development are less studied, even though embryos are particularly sensitive to abiotic conditions that are altered by global change (e.g. temperature). In reptiles, relatively warm incubation temperatures increase developmental rate and often enhance fitness-relevant phenotypes, but extremely high temperatures cause death. Due to the urban heat island effect, human-altered habitats (i.e. cities) potentially create unusually warm nest conditions that differ from adjacent natural areas in both mean and extreme temperatures. Such variation may exert selection pressures on embryos. To address this, we measured soil temperatures in places where the Puerto Rican crested anole lizard (*Anolis cristatellus*) nests in both city and forest habitats. We bred anoles in the laboratory and subjected their eggs to five incubation treatments that mimicked temperature regimes from the field, three of which included brief exposure to extremely high temperatures (i.e. thermal spikes) measured in the city. We monitored growth and survival of hatchlings in the laboratory for 3 months and found that warmer, city temperatures increase developmental rate, but brief, thermal spikes reduce survival. Hatchling growth and survival were unaffected by incubation treatment. The urban landscape can potentially create selection pressures that influence organisms at early (e.g. embryo) and late life stages. Thus, research aimed at quantifying the impacts of urbanization on wildlife populations must include multiple life stages to gain a comprehensive understanding of this important aspect of global change.

KEY WORDS: *Anolis*, Embryonic development, Global change, Heart rate, Plasticity, Thermal adaptation, Urbanization

INTRODUCTION

Human-modified habitats create novel conditions to which organisms must acclimate or adapt to survive. Urban habitats alter patterns of behavior (Lowry et al., 2013), influence rates of mortality (Koenig et al., 2002), increase population densities (Marzluff, 2001), and alter community assemblages (McIntyre et al., 2001). As such, urban environments have influenced the evolution of diverse taxa (microbes, plants, invertebrates and vertebrates; reviewed by Johnson and Munshi-South, 2017). Moreover, the effects of urban habitats on populations can be similar across the globe because of the relatively homogenous conditions that urbanization creates (McKinney, 2008; but see Niemelä et al., 2002). For example, urban areas are often characterized by a reduction in canopy cover and an increase in heat-absorbing surfaces (asphalt, concrete), which result in higher mean ambient temperatures and higher maximum temperatures in urban landscapes compared with adjacent rural or natural areas (i.e. the urban heat island effect; Arnfield, 2003).

Extreme temperatures in cities can differentially impact species and result in rapid thermal adaptation (Diamond et al., 2017). This is particularly important for ectotherms because their development, growth and reproduction are dependent upon environmental temperature. Experimenters often quantify the thermal tolerance (e.g. critical thermal maximum) or thermal performance of adults to demonstrate adaptive responses to novel thermal conditions (Angilletta et al., 2007; Diamond et al., 2017); however, few studies quantify the effects of the urban heat island on embryonic development and early life stages (Kaiser et al., 2016; Tiatragul et al., 2017). These stages are important to consider for three reasons. First, early life stages are extremely sensitive to environmental fluctuations and often have a narrower thermal tolerance than later stages (Pörtner et al., 2017). As such, abiotic conditions during embryonic development can have lasting effects on fitness-relevant phenotypes (Deeming and Ferguson, 1991; Warner, 2014; Kaiser et al., 2016). Second, because most ectotherms lay eggs that are immobile and cannot behaviorally compensate for adverse conditions, embryos (more so than adults) may be subjected to novel thermal regimes and novel selection pressures in urban environments. Although embryonic development can adapt or acclimate to local environments (Angilletta et al., 2004; Du et al., 2010a,b), little is known about how embryos respond to extreme, but brief, spikes in incubation temperature like those in urban habitats. Finally, the thermal sensitivity of embryos has the potential to influence population dynamics and species distributions (Carlo et al., 2018), so understanding how embryos respond to extreme thermal stress is essential to predict how aspects of global change (e.g. urbanization, climate change) will have an impact on population or species survival.

Anole lizards are excellent models for studying urban adaptation and acclimation because their development, growth, behavior and reproduction are correlated with abiotic conditions that vary between urban and natural landscapes (e.g. temperature, moisture, structural complexity; Reedy et al., 2012; Kolbe et al., 2016; Tiatragul et al., 2017). Moreover, they have successfully invaded urban environments both within and outside their native ranges on multiple occasions, and past studies show that urban habitats can alter their behavior, physiology, reproduction and morphology (Kolbe et al., 2016; Winchell et al., 2016; Chejanovski et al., 2017; Hall and Warner, 2017; Tiatragul et al., 2017). Existing studies, however, are biased towards adult male lizards because they are relatively easy to locate, observe and capture due to their larger size,

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Received 27 March 2018; Accepted 15 May 2018

site fidelity and conspicuous breeding behaviors. Thus, few studies have addressed the influence of urban environments on earlier life stages, like embryonic development or hatchlings (Hall and Warner, 2017; Tiatragul et al., 2017).

Like most reptiles, anoles nest in the ground, so embryos are subjected to substantial and often unpredictable environmental variation during development. Soil temperature and moisture content, which can differ markedly between urban and natural areas, have significant effects on embryo development and, thus, hatchling characteristics (i.e. growth rate, body mass and sprint speed; Reedy et al., 2012; Pearson and Warner, 2016). Little research, however, has been devoted to understanding how fluctuations or irregularities in incubation temperature contribute to patterns of embryonic development in ways that affect offspring phenotypes (e.g. Du and Ji, 2006; Les et al., 2009; Warner and Shine, 2009). Most of the work in this field has utilized constant temperature incubation (Bowden et al., 2014). This is a considerable drawback because irregular thermal events are characteristic of most nests, affect patterns of development (e.g. Angilletta et al., 2000; Warner and Shine, 2009; Carter et al., 2018), and are more common and extreme in urban areas (Tiatragul et al., 2017). Moreover, recent theoretical work predicts that thermal extremes, even when rare, can have more influence on the evolution of thermal performance than mean conditions (Buckley and Huey, 2016). Thus, studies of extreme temperatures (as opposed to 'typical' variation) are essential to understand the evolution of thermal tolerance (Buckley and Huey, 2016).

To address these issues, we conducted an egg incubation experiment to quantify how embryos from natural and urban areas respond to extreme, but brief, spikes in incubation temperature. We bred lizards from city and forest populations and subjected their eggs to incubation regimes that included brief, but extremely high thermal spikes measured from the field. We made two, non-mutually exclusive predictions. First, embryos from city populations may have better hatching success and/or higher post-hatching growth and survival than those from forest populations when exposed to brief, thermal spikes (i.e. city embryos are adapted to city conditions). Second, regardless of population of origin (city versus forest), incubation at relatively warm, city temperatures may enhance survival and hatchling traits when subjected to a thermal spike (i.e. embryos acclimate to city conditions). This work provides an ecologically meaningful and novel evaluation of developmental adaptation and plasticity and advances our understanding of their roles in urban invasion and establishment.

MATERIALS AND METHODS

Lizard collection and husbandry

Methods were approved by the Auburn University Institutional Animal Care and Use Committee (2015-2785), and specimens were deposited into the Auburn University Museum of Natural History. We collected adult crested anoles [*Anolis cristatellus* Duméril & Bibron 1837; females ≥36 mm snout–vent length (SVL); males ≥45 mm SVL] from one urban habitat (Red Road, henceforth 'city') and one forested habitat (Matheson Hammock Park, henceforth 'forest') in Miami, FL, USA. These sites are approximately 1.5 km apart. Matheson Hammock is a large fragment of dense forest and is structurally and thermally different from adjacent urban areas (Fig. S1). From 29 April to 4 May 2016, we collected adult lizards from the forest (48 females, 20 males) and city (40 females, 23 males) by hand or noose. We used data from Tiatragul et al. (2017) to determine our sample sizes (i.e. number of lizards required to produce an adequate sample of eggs). Individuals were visually sexed per body size, dewlap size and color, and the presence of post-anal scales in males. These animals were transported to Auburn University to form a captive breeding colony.

We housed females in single cages (29×26×39 cm; height×width×depth) illuminated with Reptisun 5.0 UVB and Tropic Sun 5500K daylight bulbs (Zoo Med Inc., San Luis Obispo, CA, USA) with a 12 h:12 h light:dark cycle and maintained an ambient room temperature of 25.6°C. Due to light sources, ambient cage temperatures were approximately 2–3°C higher than room temperature and, during the day, maximum daily temperatures were 31–33°C in the warmest part of the cage. Cages included two bamboo perches, an artificial plant, a nesting pot (plant pot filled with a mixture of soil and peat moss) and reptile cage carpet (Zoo Med Inc.) as a floor substrate. We fed lizards three crickets each (dusted with vitamins and calcium) three times per week and misted cages with water daily.

Because we had half as many males as females, each male was shared by two females and was rotated between them approximately once every 2 weeks. We paired males and females haphazardly, but individuals were not mixed between sites (i.e. males from the city were kept with females from the city).

Egg collection and treatment allocation

From 5 May to 23 September, we collected freshly laid eggs from nest pots three times each week. For each egg (forest population, N=179; city population, N=217), we recorded the mass (to 0.0001 g), date of oviposition and maternal identity (ID). We placed each egg in individual Petri dishes (60 mm ×15 mm) filled with moist vermiculite (−150 kPa) and wrapped the dish with Parafilm to prevent evaporation. This ensured that the ambient humidity available to eggs was controlled across treatments. Eggs were then allocated to one of five incubation treatments that mimicked thermal regimes of potential nest sites in the forest and city. Because *Anolis* lizards produce one egg every 7–10 days, each female's first egg was randomly assigned to a treatment and each successive egg she produced was assigned to one of the remaining treatments until she had at least one egg in each treatment. For females that laid more than five eggs (N=35), the sequence of treatment allocation was repeated. This minimized potential biases from the order of egg production or maternal identity.

Creation of incubation treatments

Although little is known about the nesting ecology of anoles, they usually dig shallow nests in the soil (<4 cm deep) or place eggs between the soil surface and cover materials (e.g. leaves, logs, pieces of bark; Rand, 1967). In March 2014, we placed temperature loggers (iButtons; N=5 in the forest, N=5 in the city) in locations likely to be used by anoles for nesting according to our own experience and data reported by Rand (1967). Each was placed at ∼4 cm depth in the soil. The canopy openness over these locations was 20–90% (mean=42%) in the city and 1–10% (mean=5%) in the forest. This was representative of the relatively open versus closed canopies of the city and forest, respectively.

For the city and forest, separately, we calculated the average soil temperature for each hour of the day from 1 May to 15 September (when most eggs are produced; Hall and Warner, 2017). These two thermal profiles (henceforth 'city profile' and 'forest profile'; Fig. 1A) represent average diel temperature cycles for the forest and city and were programmed into incubators (Memmert IPP55 plus) to loop daily for the duration of the study. These each served as control treatments. In addition, we selected the warmest diel fluctuation (i.e. thermal spike; 43°C peak) recorded across all city temperature loggers (Fig. 1B) and programmed an incubator to

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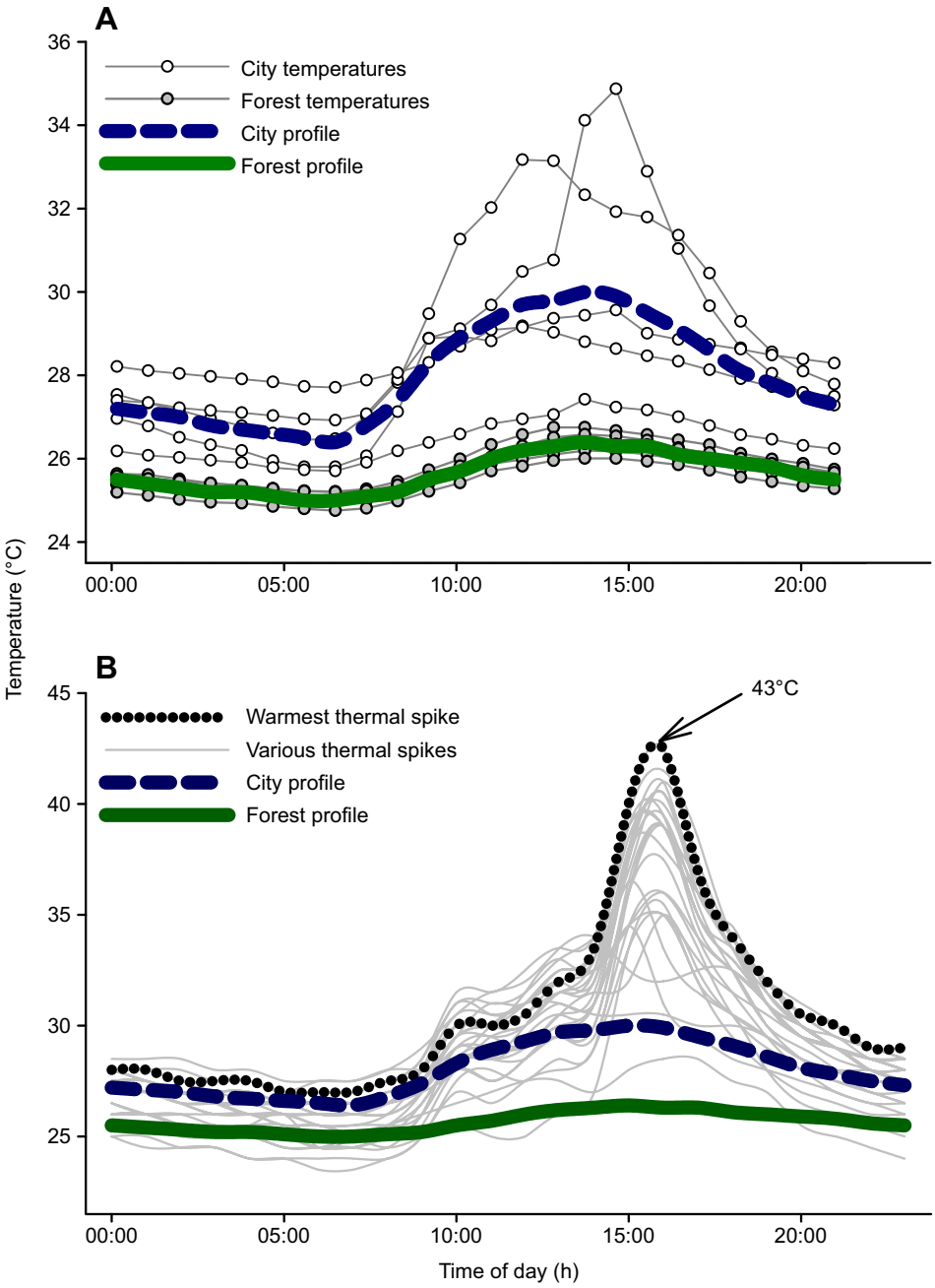

Fig. 1. Thermal data from various potential *Anolis cristatellus* nest sites in city and forest habitats. Data collected from 1 May to 15 September 2014 (five city sites and five forest sites). (A) Average hourly temperatures of nests from the city (open symbols) and nests from the forest (filled symbols). Blue and green lines represent the mean of all nests from each habitat and served as our city and forest control incubation profiles, respectively. (B) Range of various daily thermal spikes from city nests (light grey lines). Each grey line is a single daily fluctuation from a nest. The highest thermal spike (43°C peak) recorded across all potential nests was used in our study and is denoted by a string of filled circles. The city and forest incubation profiles are shown again in B to demonstrate contrast between the typical daily fluctuations and a thermal spike.

repeat this fluctuation daily. For a final incubator, we lowered the peak of this thermal spike by 4°C (the difference between the peak temperatures of the forest and city profiles) and looped this fluctuation daily to assess the effect of a lower-magnitude thermal spike (39°C peak). Thus we used four incubators: one looping the city profile, one looping the forest profile, one looping a thermal spike that peaked at 43°C, and one looping a thermal spike that peaked at 39°C.

A more recent study conducted in 2017 confirmed that our incubation regimes represent the thermal conditions of actual anole nests at our sites. In this study, mean daily nest temperatures ranged from 25.3 to 32.6°C in the city (N=43 nests) and from 24.4 to 28.6°C in the forest (N=43 nests). The warmest temperatures recorded in city and forest nests were 39.5 and 33°C, respectively (S. Tiatragul, J. M. Hall, N. G. Pavlik and D. A. Warner, unpublished data).

Experimental design

With these four incubators, we created five incubation treatments: two controls and three experimental groups. One control group was exposed to the forest profile repeated daily throughout development, and a second was exposed to the city profile repeated daily (i.e. neither control had a thermal spike; Fig. 1A). One experimental group was exposed to the city profile but given a brief thermal spike of 43°C on a single day during development (Fig. 2A). A second group was exposed to the forest profile and given a brief thermal spike of 43°C on a single day during development (Fig. 2B). These four groups allowed us to determine if habitat-specific incubation temperatures (city versus forest profile) influence an embryo's response to thermal spikes. Although the height of the thermal spike (magnitude: 43°C) was the same for both experimental groups, the differential between the thermal profiles and the peak of the thermal spike was not (Fig. 2A,B). Therefore, we created a third experimental group to be incubated at the

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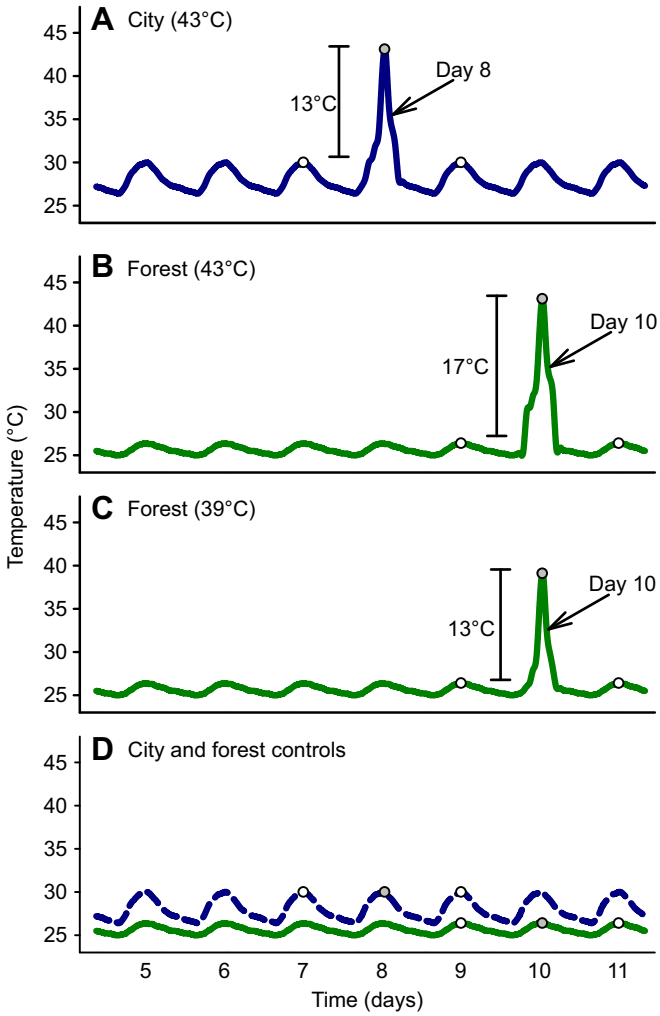

Fig. 2. Diagrams representing our five incubation treatments. Blue and green colors denote treatments that utilize the city or forest incubation profile, respectively. The temperatures in parentheses are the peak of each thermal spike. One treatment (A) consisted of the city regime looped daily and a thermal spike delivered on day 8 of development. Two treatments (B,C) consisted of the forest regime looped daily and a thermal spike delivered on day 10 of development. Because developmental rate increases with temperature, thermal spikes were delivered 2 days later to eggs incubated at the forest temperatures than those incubated at city temperatures. This ensured that all embryos received the treatment at the same stage of development. Bars show the differential between the peak of the thermal regime and the peak of the thermal spike. Embryo heart rates were recorded for one subset of eggs at the peak temperature of each spike (filled grey circles) and for another subset on the day before and the day after a thermal spike (open circles). Two control treatments (D) consisted of either the forest (continuous line) or city (dashed line) regime repeated daily throughout the entirety of incubation (no thermal spike).

forest profile and given a brief thermal spike of 39°C (Fig. 2C). Comparing this group with the others allowed us to make inferences about the relative effect of a spike's magnitude versus its differential. Each of these five groups (see Table 1 for sample sizes) consisted of eggs laid by females from both the city and forest; therefore, this 2×5 (population×treatment) factorial design allowed us to evaluate how embryos from different populations respond to habitat-specific incubation conditions and thermal spikes.

All eggs were placed into the incubator that looped their assigned thermal profile (city or forest). On the appropriate day (see below), we moved experimental eggs from these incubators to their assigned treatment incubator (either 39 or 43°C thermal spike). Control eggs were moved within the incubator (i.e. from one shelf to another) to account for any effect of moving eggs. Each egg remained in this position until the following day when eggs were returned to their original positions for the remainder of incubation. These manipulations were made each day prior to 09:00 h when the thermal spike began (Fig. 1B). Eggs were incubated at their assigned incubation profile for the remainder of development (see Fig. 3 for design summary).

Because developmental rate increases with temperature, thermal spikes were delivered 2 days later (i.e. day 10 of development) to eggs incubated at the cooler, forest profile than those incubated at the city profile (day 8). This ensured that all embryos experienced the treatment at approximately a quarter of the way through egg incubation. We estimate this is embryo stage 8 or 9 based on published data of developmental rates from Tiatragul et al. (2017) and the *Anolis* embryo staging series of Sanger et al. (2008). We chose this period to give embryos time to potentially acclimate to their respective incubation regime (i.e. city or forest) yet expose them to the spike relatively early in development. Previous work on lizards shows that this early time is highly sensitive to temperature (Shine and Elphick, 2001; Andrews, 2004).

Heart rate detection

To determine the immediate and lasting effects of thermal spikes on embryo physiology, we non-invasively measured heart rates using the Buddy egg monitoring system (https://www.avitronics.co.uk/) (Du et al., 2009). This device uses infrared light to detect the heart rate of small embryos. For a subset of eggs (N=84) we measured heart rates at the peak of each thermal spike (henceforth 'stressed heart rate'), and for another subset (N=62) we used a repeated measures design to measure heart rates 24 h before and 24 h after a thermal spike (henceforth 'resting heart rate'). To measure heart rates, we quickly removed eggs from the incubator and placed them into the Buddy (at room temperature) and obtained heart rate measures within 20–30 s. Some eggs had to be repositioned to obtain a heart rate, so we recorded the number of repositions (0, 1 or 2) and included this as a covariate in our analyses. These measurements have no effect on developmental rate or survival of anole embryos (Hulbert et al., 2017).

Hatchling measurement and husbandry

We recorded the hatch date, SVL and tail length (to the nearest 1 mm using a ruler) and body mass (to the nearest 0.0001 g) of each hatchling (see Table 1 for sample sizes) and housed them in the laboratory for approximately 3 months (mean 84.3 days; standard deviation 12.18) to monitor growth and survival. Hatchlings were uniquely toe-clipped for identification and housed in cages with conditions identical to those described for adults except that there was no nesting pot, and we provided additional foliage (artificial plants) to increase surface area for perching and hiding. Intraspecific competition is an important determinant of growth and survival in *Anolis* lizards (Calsbeek and Cox, 2010), so we housed hatchlings communally: six lizards per cage. Hatchling densities in the field are high (Lee et al., 1989) and comparable to our housing conditions (J.M.H. and D.A.W., personal observation). As eggs incubated at the city profile hatched sooner than those incubated at the forest profile, we segregated hatchlings according to incubation profile to prevent large discrepancies between the oldest and youngest individuals in each cage. Thus, we had two types of cages: some contained three lizards from the city control group and three from the city experimental group (six total lizards per cage; N=9 cages) while others contained two lizards from

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Table 1. Sample size, mean and standard deviation of raw data for measures of development, physiology and morphology in *Anolis cristatellus*

	Forest		City		Forest (39°C)		Forest (43°C)		City (43°C)	
Dependent variable	*N*	Mean±s.d.	*N*	Mean±s.d.	*N*	Mean±s.d.	*N*	Mean±s.d.	*N*	Mean±s.d.
Egg										
Survival (%)	79	67	81	67	78	68	74	55	76	43
Incubation period (days)	54	42.73±2.23	54	34.85±2.12	53	43.31±2.37	41	44.02±2.44	33	35.71±1.82
Stressed heart rate (beats min^{-1})	21	94.14±9.67	17	101.65±16.41	16	149.44±43.42	14	166.93±44.59	16	175.31±45.64
Resting heart rate (beats min^{-1})	19	−3.68±7.58	17	−2.00±21.85	7	−8.29±12.46	8	−12.75±7.85	11	−12.09±16.97
Hatchling										
Survival (%)	40	46	27	44	40	36	40	50	27	31
Initial mass (g)	54	0.2288±0.0283	54	0.2284±0.0367	53	0.2235±0.0344	41	0.2315±0.0312	33	0.2359±0.0269
Initial SVL (mm)	54	19.34±1.02	54	19.29±1.11	53	19.34±0.99	41	19.27±0.84	33	19.24±0.90
Initial body condition	54	−0.0001±0.10	54	0.0006±0.13	53	−0.03±0.12	41	0.008±0.11	33	0.035±0.10
Initial tail length (mm)	54	28.92±1.6	54	28.94±1.63	53	28.62±1.98	41	28.32±1.6	33	28.3±2.26
Final mass (g)	19	0.6020±0.1950	12	0.5357±0.1424	14	0.5439±0.1494	20	0.5057±0.1681	8	0.5620±0.1983
Final SVL (mm)	19	25.95±2.84	12	24.83±2.12	14	24.43±1.60	20	24.25±2.34	8	25.13±2.95
Final body condition	19	−0.032±0.13	12	0.002±0.13	14	0.054±0.14	20	−0.002±0.15	8	−0.018±0.14

Because we observed no population (city versus forest)×incubation treatment interactions, we combined data from both populations to generate these means. Resting heart rate is the mean difference between the heart rates measured 1 day before and 1 day after a thermal spike (after minus before).

the forest control, and two from each of the two forest experimental groups (six total lizards per cage; N=20 cages). We assigned hatchlings to cages in order of hatching. Because of differential egg survival among groups, we produced an excess of hatchlings from some treatments, and some lizards (N=61) were euthanized by decapitation after hatching. These were systematically chosen throughout the study in a way that minimized age discrepancies among cage-mates. Although our cage assignments are not random, we created ecologically relevant housing for hatchlings that minimized biases due to order of hatching (thus age and size).

We misted cages with water daily and fed lizards appropriately sized crickets dusted with calcium and vitamins. We checked each cage for dead lizards prior to every feeding to ensure that, despite variation in the number of surviving lizards across cages, prey density was held constant through the study (four crickets per lizard, three times each week). When all lizards in a cage had reached approximately 3 months of age, we noted survival and remeasured their SVL and body mass. Each lizard was then euthanized by injection of sodium pentobarbitol.

Statistical analyses

We used generalized linear mixed effects models to analyse egg and hatchling survival, and mixed effects linear models to analyse incubation period, heart rate and body size (SVL, tail length, body condition and mass). Initial hatchling body condition was a residual score from a second-degree polynomial regression of log mass versus log SVL (Schulte-Hostedde et al., 2005; but see also Peig and Green, 2010). Final hatchling body condition was calculated without the second-degree term because adding it did not improve fit (F_1=1.11; P=0.3). Each analysis included incubation treatment, population (city versus forest), and their interaction as fixed effects. We also included covariates when appropriate (see Table 2). We built two models for each analysis: one with maternal ID as a random effect and one without. We used likelihood ratio tests or chi-squared tests to determine which model best fitted the data (Table 3). These analyses assessed the potential for maternal effects (e.g. genotype) to influence dependent variables; however, regardless of their outcome, we report the results from the model that included maternal ID (to avoid pseudo-replication of the population variable). For models of hatchling survival and measures of final body size, we also included hatchling cage as a random effect; thus, maternal ID and cage were modeled as crossed random effects. Because hatchlings in each cage varied in age, we assigned the final lizard added to each cage an age of 1 day and each other lizard in that cage was given an age according to how many days it had been in the cage prior to the addition of the final lizard. We included this relative age in our model.

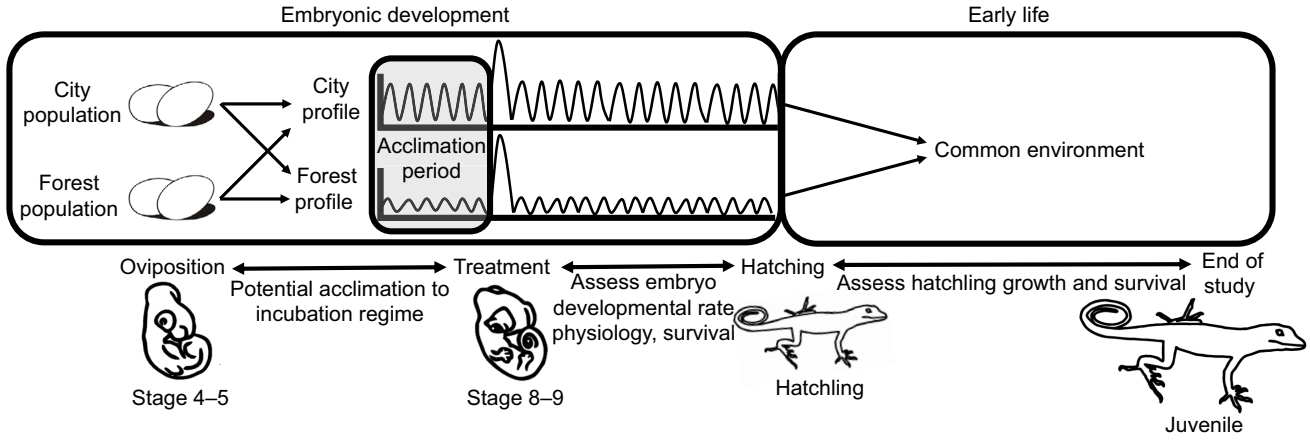

Fig. 3. Overview of our design to measure the effects of city and forest thermal regimes on embryonic and early life stages of lizards from both city and forest populations. Thermal treatments were applied approximately a quarter of the way through development. See Fig. 2 and Materials and Methods for more details concerning the thermal treatments. Embryo staging is based on Sanger et al. (2008).

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Table 2. Effect of incubation treatment, population (city versus forest) and their interaction on embryonic development, physiology and hatchling morphology

	Incubation treatment			Population			Interaction		
Dependent variable	d.f.	F or χ^2	P	d.f.	F or χ^2	P	d.f.	F or χ^2	P
Egg									
Survival[a]	4	10.47	**0.033**	1	1.89	0.17	4	1.56	0.82
Incubation period[a]	4,176	119.74	**<0.0001**	1,47	0.83	0.37	4,176	0.44	0.78
Stressed heart rate[b]	4,40	11.31	**<0.0001**	1,33	0.17	0.69	4,40	0.22	0.93
Resting heart rate[b]	4,52	19.42	**<0.0001**	1,52	0.34	0.56	4,52	2.49	0.055
Hatchling									
Survival[c,d]	4	3.3	0.51	1	0.23	0.64	4	3.55	0.47
Initial mass[a]	4,176	1.95	0.10	1,47	1.02	0.32	4,176	1.84	0.12
Initial SVL[a]	4,176	0.32	0.86	1,47	0.48	0.49	4,176	0.28	0.89
Initial body condition[a]	4,176	1.09	0.36	1,47	1.83	0.18	4,176	1.28	0.28
Initial tail length[a]	4,176	1.94	0.11	1,47	0.18	0.68	4,176	0.49	0.74
Final mass[c,d]	4,42	1.15	0.35	1,27	0.99	0.33	4,49	2.24	0.08
Final SVL[d,e]	4,50	2.25	0.08	1,28	2.94	0.10	4,50	2.39	0.06
Final body condition[d,f]	4,51	1.41	0.24	1,34	2.24	0.14	4,52	1.77	0.15

Covariates: [a]egg mass, [b]trials, [c]initial body mass, [d]relative age, [e]initial SVL, [f]initial body condition. Sample sizes, means and standard deviations are given in Table 1. Statistics for each covariate are given in Table S1, and graphs are in Fig. S2. Survival test statistics are χ^2, others are F-values. Values in bold denote statistical significance.

The range of relative ages was 1–31 days and the average was 7.29 days. Most lizards (90.3%) had a relative age of less than 15 days. For stressed heart rate, we performed a Box–Cox transformation to account for heteroskedasticity. To achieve model convergence for egg survival and hatchling survival, we utilized the Nelder–Mead optimizer and rescaled and centered all continuous covariates at zero (Bolker et al., 2009). For models with crossed random effects, we used the lme4 package (http://cran.us.r-project.org/web/packages/lme4) and then generated P-values with the lmerTest package (https://cran.r-project.org/web/packages/lmerTest). This package calculates denominator degrees of freedom by Satterthwaite approximation like SAS proc mixed theory. Analyses were performed in R 3.1.3 (http://www.R-project.org/).

RESULTS

Including maternal ID as a random effect significantly improved model fit for egg survival, initial hatchling mass, initial hatchling tail length, and final hatchling mass and SVL (Table 3); however, we observed no differences between city and forest populations for survival, development or hatchling traits (Table 2). We also found no interactions between incubation treatment and population: eggs and hatchlings from the city and forest responded similarly to all treatments both before and after hatching (Table 2). The incubation treatment, however, significantly influenced egg survival, incubation period and embryo heart rate, but had no effect on the survival, morphology or growth of hatchlings (Table 2). Egg survival decreased by as much as 24% when eggs were exposed to our most extreme thermal spike (43°C), but the lower spike (39°C) had no effect (Fig. 4A; Table 1). Eggs incubated at cooler forest temperatures had an incubation period 7.87±0.39 days (mean±s.e.m.) longer than eggs incubated at city temperatures (Fig. 4B; Table 1). Thermal spikes had minimal effect on incubation period; however, eggs from all three experimental groups took longer to hatch than their respective controls (Fig. 4B). This was only statistically significant for one group: eggs incubated at the forest profile and given a 43°C spike took 1.37±0.42 days (mean±s.e.m.) longer to hatch than the control (Table 1). Finally, the incubation treatments altered embryo physiology (Table 2). Heart rate increased substantially during a thermal spike (Fig. 5A); however, on the day following treatment, embryos that experienced a thermal spike had lower resting heart rates than controls and lower heart rates than their own pre-exposure baselines (Fig. 5B; Table 1).

Table 3. Comparisons of models with and without maternal ID as a random effect

	With maternal ID			Without maternal ID				
Dependent variable	AIC	BIC	logLik	AIC	BIC	logLik	Test statistic	P
Egg								
Survival	**510.07**	**557.61**	**−243.04**	519.86	563.43	−248.93	**11.78***	**0.001**
Incubation period	1011.04	1055.34	−492.52	1010.59	1051.47	−493.29	1.54	0.21
Stressed heart rate	−454.29	−424.51	240.14	−455.97	−428.49	239.99	0.31	0.57
Resting heart rate	872.63	923.59	−417.32	870.63	918.91	−417.32	0.01	0.99
Hatchling								
Survival	254.83	298.98	−113.42	252.97	293.97	−113.49	0.14*	0.71
Initial mass	**−930.90**	**−886.60**	**478.45**	−926.69	−885.80	475.35	**6.21**	**0.01**
Initial SVL	654.44	698.73	−314.22	652.87	693.75	−314.43	0.43	0.51
Initial body condition	−318.94	−274.65	172.47	−320.18	−279.29	172.09	0.77	0.38
Initial tail length	**926.27**	**970.57**	**−450.14**	936.02	976.91	−456.01	**11.74**	**0.001**
Final mass	**−48.1**	**−13.74**	**39.05**	−40.92	−8.85	34.46	**9.18***	**0.002**
Final SVL	**331.13**	**365.49**	**−150.57**	335.18	367.25	−153.59	**6.05***	**0.014**
Final body condition	−81.46	47.11	55.73	−80.21	−48.14	54.11	3.25*	0.07

Values in bold denote models where including maternal ID significantly improves the fit of the model. *Chi-squared test statistic. All other test statistics are log likelihood ratio statistics. AIC, Akaike's information criterion; BIC, Bayesian information criterion; logLik, log likelihood.

Journal of Experimental Biology (2018) 221, jeb181552. doi:10.1242/jeb.181552

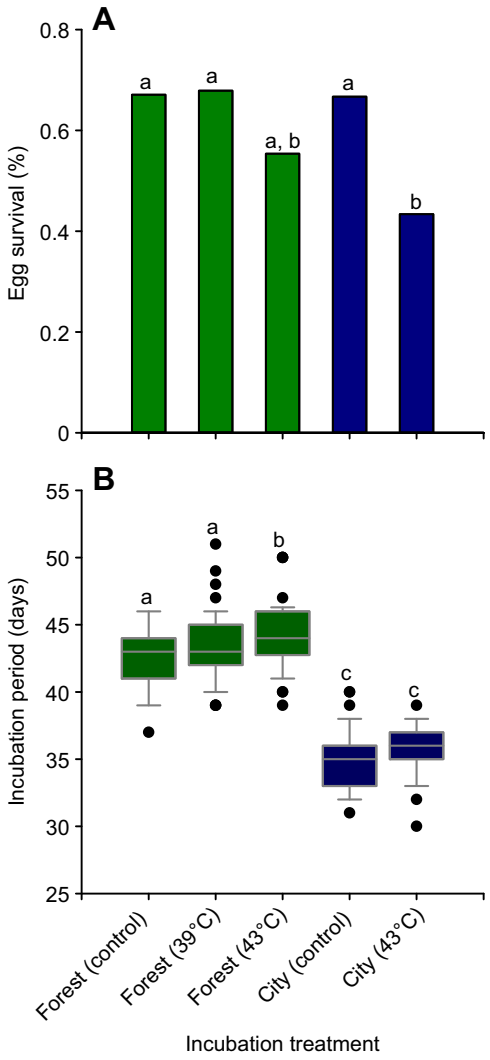

Fig. 4. Egg survival and incubation period for each treatment. (A) Egg survival; (B) incubation period. Green and blue bars denote treatments with the forest and city incubation profile, respectively. Boxes and whiskers show quartiles, horizontal lines show the median, and black circles show raw data points that are above and below the upper and lower quartiles. Lower case letters show groups that were statistically different from one another after false discovery rate correction. Because we found no interaction between population (city versus forest) and incubation treatment, we combined data from both populations for these graphs. See Tables 1 and 2 for results of statistical tests, sample sizes and estimates.

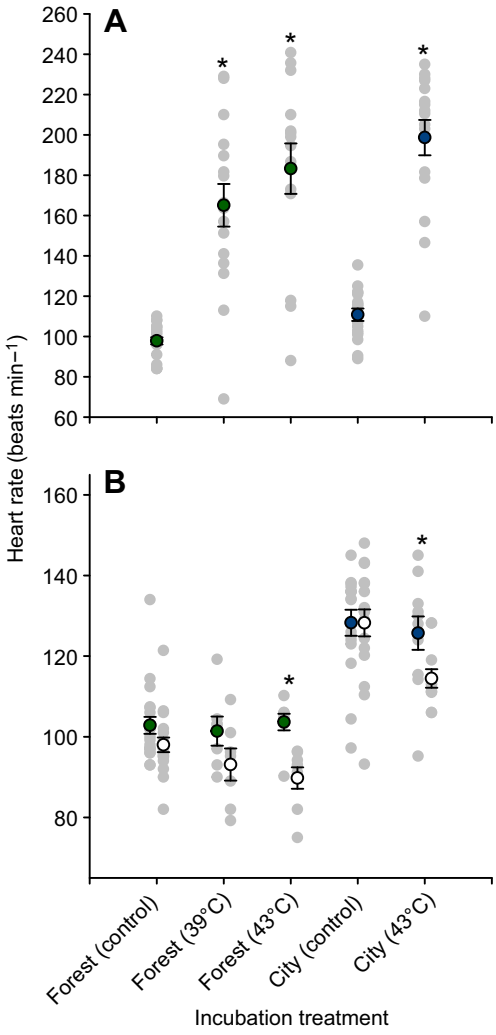

Fig. 5. Embryo heart rates before, during and after a brief thermal spike. Grey circles show raw data and bars represent standard error. Green and blue circles denote treatments with the forest and city incubation profile, respectively. (A) Mean stressed heart rates (green and blue circles) at the peak of each thermal spike. Controls received no thermal spike; thus, heart rates were recorded at the peak of a typical diel cycle. The temperatures at which heart rates were measured were 26, 30, 39, 43 and 43°C, which are the peak temperatures of each treatment listed from left to right. (B) Mean resting heart rates recorded 24 h before (green and blue circles) and 24 h after (white circles) a thermal spike. Heart rates were recorded at the peak of a typical diel cycle: 26 and 30°C for forest and city regimes, respectively. Because we found no interaction between population (city versus forest) and incubation treatment, we combined data from both populations for these graphs. Asterisks signify statistical difference between (A) an experimental group and its associated control or (B) heart rate before and after a thermal spike. *P*-values were adjusted for false discovery rate detection, and mean values are least squares means. See Tables 1 and 2 for results of statistical tests, sample sizes and estimates.

DISCUSSION

Urban environments create novel thermal conditions that differ from adjacent non-urban areas in both mean and extreme temperatures. Because early life stages (i.e. embryos, hatchlings) of reptiles are very sensitive to abiotic conditions, extreme temperatures in urban habitats may have consequences for survival, physiology, development and growth. Although embryos can adapt or acclimate to local conditions, virtually nothing is known about how they respond when briefly exposed to extreme temperatures, and even less is known about the influence of urban incubation regimes on development. Our experiment does not support the hypothesis that anole embryos have adapted to local urban incubation regimes, nor does it show evidence that embryos acclimate to urban thermal conditions. The extreme ground temperatures of urban environments, however, do have the potential to increase egg mortality and alter patterns of development.

Population-specific effects

Embryos from two different populations may differ in their response to the same developmental conditions if local embryo adaptation has occurred or if maternal effects (i.e. nesting behavior, resource allocation to eggs) influence responses to local developmental environments (these are not mutually exclusive). For example, embryo physiology and maternal nesting behavior of the eastern fence lizard (*Sceloporus undulatus*) are adapted to the cool temperatures and short reproductive season of high-latitude environments (Angilletta et al., 2004). Females in these

populations nest in warm, open canopy microhabitats, and their embryos develop more quickly at cool temperatures than those from low-latitude populations.

Relatively few studies have explored local adaptation of reptile embryos (Angilletta et al., 2004, 2013; Du et al., 2010a), and only one has explored potential adaptation to urban environments. Although Tiatragul et al. (2017) found no evidence that lizard embryos are adapted to urban thermal conditions, they did not expose embryos to the extremely high temperatures that often occur in urban areas. Reptile embryos can employ a diversity of strategies to physiologically respond to variable nest temperatures (Du and Shine, 2015); however, adaptation of reptile embryos to extremely high temperatures has not yet been observed (Angilletta et al., 2013). This may be because maximum temperatures vary less than minimum temperatures as latitude increases, and thus critical thermal maximums of adult ectotherms are often uniform throughout their range (Sunday et al., 2011). Extreme temperatures in cities, however, may be above the range of naturally occurring maximum temperatures and induce novel selection on embryo phenotypes.

Maternal effects also have the potential to shield embryos from adverse environmental conditions. For example, females can influence offspring phenotypes through differential allocation of resources to eggs (i.e. yolk or hormones), and these allocations can be environmentally dependent (e.g. maternal diet; Rutstein et al., 2004; Lovern and Adams, 2008; Warner and Lovern, 2014). Chejanovski et al. (2017) found that urban anoles can be less responsive when offered food and have greater body size and body condition when compared with non-urban congeners, indicating that food is more abundant in urban areas. Indeed, the city females used in our study had greater body size and condition than those from the forest (Hall and Warner, 2017) and may have started the breeding season with greater fat reserves to fuel reproduction. Therefore, population-specific maternal effects could potentially influence how embryos respond to brief, thermal stress.

Despite the potential for adaptation or maternal effects to influence development, embryos from both populations responded similarly to brief, thermal spikes, and so our data do not support our prediction that embryos from the city are better able to mitigate the adverse effects associated with urban thermal incubation environments than those from the forest. We suggest four, non-mutually exclusive explanations. First, the two study populations are only ∼1.5 km apart. Although separated by a canal, plenty of roads and walkways cross the water; hence gene flow between our city and forest populations could stifle local adaptation. Second, our study populations may not have had sufficient time to genetically diverge from one another. Indeed, *A. cristatellus* was first detected in South Florida in 1976, and because these lizards mature in about 1 year, approximately 40 generations have passed. This time is sufficient for local adaptation of adult phenotypes (e.g. thermal tolerances of *A. cristatellus* have adapted to the climate of south Florida; Kolbe et al., 2012; Leal and Gunderson, 2012). We do not know, however, how much time is necessary for embryonic phenotypes to adapt to local conditions. Embryo phenotypes may take longer to adapt to local conditions than adults if maternal nesting behavior is highly plastic (Doody et al., 2006).

Third, nesting behavior can potentially shield embryos from adverse environmental conditions (Doody et al., 2006; Reedy et al., 2012). Our temperature data from the field is a representation of the potential thermal landscape available for nesting females in both city and forest habitats; however, no study has yet determined how nesting behavior differs between these environments. Nesting behavior of *Anolis* lizards is poorly understood. A diversity of anecdotal field observations show that anoles utilize a variety of nesting microhabitats (i.e. leaf litter, under logs; Rand, 1967), but few studies have quantified factors associated with site selection or egg survival in the field (e.g. Andrews, 1988). If nests are exposed to thermal regimes like those in our study, extreme temperatures may reduce egg survival in the city by as much as 20%. This could be a conservative estimate as we only delivered a thermal spike on a single day during development. Eggs in cities may experience multiple thermal spikes that vary in magnitude (Fig. 1), and the compounding effects of such repetitive, acute thermal stresses are unknown. Finally, thermal spikes may be rare enough through space and time that they induce little selective pressure. Out of 615 daily thermal cycles measured in the city (123 collection days×5 collection locations), ground temperatures met or exceeded 39°C only 20 times (3.3%).

Although urban adaptation of embryos has not yet occurred, accounting for variance due to maternal ID improved model fit for egg survival and some aspects of hatchling morphology; thus, there is at least the potential for embryo adaptation to occur as some of this maternal variance is likely to be genetic. We conclude, however, that city environments impose selection on embryos to adapt to extreme temperatures, on female nesting behavior, or both. As we do not see evidence of embryo adaptation to urban incubation conditions, nest-site choice may be how lizards cope with these extreme temperatures.

Effects on embryo and hatchling survival and phenotypes

Tiatragul et al. (2017) suggest that *A. cristatellus* embryos are physiologically robust to variation in nest temperature, and, therefore, urban habitats may enhance fitness due to increased rates of embryonic development. They further speculate this may facilitate the establishment of *Anolis* lizards outside their native range as their spread can be associated with urban sprawl. Like Tiatragul et al. (2017), we found that urban incubation temperatures enhanced developmental rate at no apparent cost to hatchling morphology or survival, and we observed no population-specific effects on embryo development (i.e. no adaptation). However, we show that embryos are not robust to the urban thermal landscape when thermal spikes are considered, and, thus, the positive effects of increased developmental rate in urban nests may come at a cost to egg survival due to thermal spikes. The conflicting conclusions of their work and ours demonstrate that assessing the impact of urban conditions on wildlife is a complex task and will likely require a synthesis of various studies. In addition, multiple aspects of life history must be considered across all life stages. For example, past work shows that fecundity of lizards may be enhanced in urban areas (Lucas and French, 2012; Hall and Warner, 2017) relative to adjacent rural or natural areas; however, if egg mortality is greater in urban habitats, the combined effect of the urban landscape on reproduction and development may be a zero sum with respect to fitness.

Exposure to extreme nest temperatures may be uncommon in the field, but theoretical models demonstrate that even rare exposure to extreme temperatures can substantially alter thermal performance and sensitivity (Buckley and Huey, 2016). This may be particularly important for embryos because they have little opportunity to behaviorally compensate for extreme temperatures (Du and Shine, 2015). Although studies of incubation temperature on reptile development abound, most studies have used constant temperatures or fluctuating temperatures that do not mimic natural conditions (Noble et al., 2018). The consequences of fluctuating temperatures on development differ from those of constant temperatures, even when

mean temperature is the same (Les et al., 2009; Bowden et al., 2014). Nest temperatures in the field often briefly exceed the maximum constant temperatures that allow for successful development in the laboratory, yet there are no standard assays to quantify the effects of extreme thermal stress on reptile embryos (Angilletta et al., 2013). Given that two aspects of global change, urbanization and climate change, both ensure that extreme nest temperatures will become more common, we need a better understanding of the effects that brief, thermal spikes have on development and survival.

Effects on embryo physiology

For complex organisms like animals, the processes of ventilation and circulation are likely to set the limits of thermal tolerance: at high temperatures, the body's demand for oxygen exceeds supply (Pörtner, 2002; the oxygen- and capacity-limited thermal tolerance concept, see Pörtner et al., 2017). Reptile eggs obtain oxygen via diffusion through the shell, thus increasing heart rate is necessary to supply oxygen to tissues during heat stress (i.e. no ventilation apparatus). Reptile embryo heart rates generally increase by a factor of 1.5–2.6 for a 10°C rise in temperature (Angilletta et al., 2013), and our measurements of anole heart rates during thermal spikes are similar (Fig. 5A).

Few studies have attempted to measure the thermal tolerance of reptile embryos (e.g. Angilletta et al., 2013; Smith et al., 2015). These studies either increased incubation temperature until embryo mortality reached 100% or quantified the influence of various concentrations of atmospheric oxygen on embryo survival. Studies that use mortality as a metric for thermal tolerance or that only explore the effect of ambient oxygen supply on mortality cannot identify the underlying mechanisms that determine the thermal tolerance of embryos (e.g. metabolic depression; Pörtner et al., 2017). Thus, studies that put less emphasis on mortality and more on sub-lethal thermal constraints of respiration or metabolism will provide novel insights (e.g. heart rate; Pörtner et al., 2017). To better understand what factors are associated with survival at ecologically relevant levels of thermal stress, embryos should be pushed to their tolerance limits and given the opportunity to recover. In doing this, we discovered two subtle effects of thermal spikes on reptile embryo physiology: developmental rate and heart rate were depressed in embryos that survived a thermal spike.

Compared with respective controls, incubation period was slightly longer for all three experimental groups, although only one was statistically significant (Fig. 4B; Table 1). This could be due to differential survival of embryos based on metabolic rate. Embryos with relatively high metabolic rates, and thus higher developmental rates, may exhibit greater oxygen consumption and be more likely to die during a thermal spike due to hypoxia (Smith et al., 2015). Conversely, this slight increase in incubation period may be due to metabolic depression induced by thermal stress, as thermal spikes reduced heart rate by ∼10% (Fig. 5B). This heart rate depression was not due to differential mortality because we utilized a repeated measures design and any embryos that were killed during the thermal spike were not included in our analysis. If thermal spikes result in reduced heart rates for 1 or 2 days, this would correspond with a subsequent increase in incubation period as incubation period negatively co-varies with heart rate (Du et al., 2009, 2010a).

The biological significance of these physiological effects is difficult to assess. An increase in incubation period of 1 day is probably not ecologically meaningful, especially in comparison with the 8-day decrease in incubation period caused by the city temperatures generally (Table 1). Furthermore, because we observed no adverse effects of thermal spikes on post-hatching growth or development, we have no reason to assume that the observed changes in physiology were permanent or detrimental.

Although past work demonstrates a strong relationship between heart rate and metabolic rate for lizard embryos (Du et al., 2010b), no study has defined this relationship for *Anolis* lizards. Thus, we will not speculate about the absolute impact of reduced heart rates on metabolism, but rather, merely assert that our data show that heart rate is reduced in anole embryos for at least 24 h after a thermal spike. Although the consequences reported here might seem insignificant, the effect could accumulate if embryos are repeatedly exposed to thermal spikes during development. Indeed, in reptiles with temperature-dependent sex determination (e.g. turtles), repeated, extreme thermal fluctuations at high temperatures can increase the incubation period and reverse sex ratios because developmental rates are depressed at extremely high temperatures (Neuwald and Valenzuela, 2011). Our data, however, compel us to consider that development may not only slow during exposure to such extreme fluctuations but also for some time after exposure. Further studies are needed to determine how long the heart rate depression we observe may last and the extent to which it corresponds with changes in metabolic rate or survival. In other animals, metabolic depression can mitigate the adverse effects of thermal stress (Guppy and Withers, 1999; Pörtner et al., 2017); however, this has never been considered for reptile embryos.

Potential acclimation to urban incubation temperatures

Reptile embryos can acclimate to local nest conditions (Du and Shine, 2015). Du et al. (2010b) found that embryos have relatively high metabolic rates when acclimated to cool temperatures, allowing them to partially compensate for the reduction in developmental rate caused by cooler incubation temperatures. We do not yet know, however, if such acclimation can ameliorate the adverse effects of extreme fluctuations in nest temperature. As hypoxia is a likely cause of death at extremely high temperatures, we predicted that embryos incubated at cooler, forest temperatures would have relatively higher metabolic rates due to acclimation than those incubated at warmer, city temperatures and would, thus, be more susceptible to death during a thermal spike because of their relatively greater oxygen consumption. Embryos incubated at city temperatures would, therefore, better mitigate the adverse effects of urban, thermal spikes. We observed the opposite trend: embryos incubated at city temperatures were least likely to survive a thermal spike (Fig. 4A). Likewise, recent studies show that higher incubation temperatures can actually lower the thermal tolerance of early life stages and result in poorer performance and increased mortality under thermal stress (Dayananda et al., 2017). Much of this research is intended to assess the vulnerability of ectotherms to climate change; however, changes in temperature due to climate warming may be comparable to those caused by urbanization (Youngsteadt et al., 2015). We think that further study of this phenomenon is warranted. The adverse effects of high incubation temperatures in urban areas are likely to be exacerbated by future climate change, and the ability for females to ameliorate these effects through nesting behavior is limited (Telemeco et al., 2009).

Conclusion

Although we show that thermal spikes caused by the urban heat island effect have the potential to reduce egg survival, we did not detect any evidence that embryos are adapted to urban incubation conditions. We also found no evidence that embryos can acclimate to urban incubation temperatures in ways that mitigate the risks associated with extremely high temperatures. However, we show

that brief thermal spikes have the potential to reduce egg survival and alter embryo physiology both during and at least 24 h following exposure. Although past work demonstrates that urban conditions have positive impacts on body size, reproduction and embryo development, extreme fluctuations in incubation temperatures may reduce egg survival and, thus, fitness. Most studies of urban adaptation have been conducted on adults, but we emphasize that studies focused on multiple aspects of life history across all life stages will better assess the impact of urbanization on wildlife. A synthesis of such work would create a more accurate picture of the various ways that global change influences evolutionary processes.

Acknowledgements
We thank D. Douglas, A. Hulbert, L. McCoy, C. Guiffre, R. Lloyd and T. Mitchell for help with animal care, J. Stroud for help collecting animals, J. Kolbe for collecting iButton data and M. Wolak for help with statistics. This is contribution no. 869 of the Auburn University Museum of Natural History.

Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: D.A.W.; Methodology: J.M.H., D.A.W.; Formal analysis: J.M.H.; Resources: D.A.W.; Writing - original draft: J.M.H.; Writing - review & editing: D.A.W.; Visualization: J.M.H.; Supervision: D.A.W.; Project administration: D.A.W.; Funding acquisition: J.M.H., D.A.W.

Funding
Research was funded by the Alabama Academy of Sciences and the National Science Foundation (DEB-1564563).

Supplementary information
Supplementary information available online at
http://jeb.biologists.org/lookup/doi/10.1242/jeb.181552.supplemental

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