RESEARCH ARTICLE

Effects of night-time warming on temperate ectotherm reproduction: potential fitness benefits of climate change for side-blotched lizards

Donald N. Clarke[1] and Peter A. Zani[1,2,*]

[1]Department of Biology, Whitman College, Walla Walla, WA 99362, USA and [2]Department of Biology, Gonzaga University, Spokane, WA 99258, USA

*Author for correspondence at present address: Department of Biology, Pomona College, Claremont, CA 91711, USA (bichodopei@yahoo.com)

SUMMARY

Temperate ectotherms, especially those at higher latitudes, are expected to benefit from climate warming, but few data yet exist to verify this prediction. Furthermore, most previous studies on the effects of climate change utilized a model of uniform annual change, which assumes that temperature increases are symmetric on diurnal or seasonal time scales. In this study, we simulated observed trends in the asymmetric alteration of diurnal temperature range by increasing night-time temperatures experienced by female lizards during their ovarian cycle as well as by the resulting eggs during their incubation. We found that higher night-time temperatures during the ovarian cycle increased the probability of reproductive success and decreased the duration of the reproductive cycle, but did not affect embryo stage or size at oviposition, clutch size, egg mass or relative clutch mass. Furthermore, higher incubation temperatures increased hatchling size and decreased incubation period but had no effect on incubation success. Subsequent hatchlings were more likely to survive winter if they hatched earlier, though our sample size of hatchlings was relatively small. These findings indicate that higher night-time temperatures mainly affect rate processes and that certain aspects of life history are less directly temperature dependent. As our findings confirm that climate warming is likely to increase the rate of development as well as advance reproductive phenology, we predict that warmer nights during the breeding season will increase reproductive output as well as subsequent survival in many temperate ectotherms, both of which should have positive fitness effects.

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Key words: asymmetric warming, diurnal temperature range, incubation, phenology, ovarian cycle, overwinter survival.

INTRODUCTION

Anthropogenic climate change has the potential to affect many organisms as global temperatures rise. Global annual average temperatures have increased steadily in recent decades (0.2°C per decade), but estimates of average warming over the next century vary from 1.1–2.9°C (scenario B1: smooth transition from fossil fuels, balanced economic growth) to 2.4–6.4°C (scenario A1F1: fossil fuel intensive, rapid economic growth) (IPCC, 2007). Regardless, climate warming has already produced observable effects on the distribution, abundance and evolution of species (Bradshaw and Holzapfel, 2006; Parmesan, 2006). In the light of these responses, accurately predicting the future impact of climate change on organisms is a major concern to biologists. Most studies analyzing effects of global warming have focused on broad-scale changes in mean temperature (Buckley, 2010), but examining global average temperature trends overlooks regional variations in climatic changes. Specifically, temperatures are rising more rapidly at mid- to high-latitudes (IPCC, 2007). In addition, observed temperature increases are asymmetrically distributed over diurnal time frames, with daily minima increasing at a faster rate than daily maxima (DeGaetano and Allen, 2002; Easterling et al., 1997; Easterling et al., 2000; Karl et al., 1991; Karl et al., 1993). Furthermore, the largest changes in diurnal temperature range appear to have occurred in the winter and the smallest changes in the summer (Easterling et al., 1997). While there is great value in broadly applicable models

of climate effects generated using annual average temperatures, such studies lack the specificity to predict changes in life-cycle processes caused by regional, seasonal or diurnal asymmetry in temperature change. The biological relevance of asymmetric warming should not be ignored but remains largely unstudied. The purpose of this study was to determine whether a night-time temperature increase alone is enough to evoke life-history responses in ectotherms.

Recent research has demonstrated that both short- and long-term temperature variations can have significant effects on biotic interactions. For example, the degree of daily temperature fluctuation impacts the infection and spread of disease (Paaijmans et al., 2010; Lambrechts et al., 2011). Similarly, climatic variability associated with natural dynamic modes, such as the El Niño–Southern Oscillation, has been implicated in the decline of amphibian biodiversity due to chytrid fungus (Rohr and Raffel, 2010). In addition, decreases in diurnal temperature variation associated with rising nocturnal temperatures affect growth and developmental rates in plants (Constable and Retzlaff, 2000) and insects (Whitney-Johnson et al., 2005). In this study, we sought to extend our understanding of the impact of asymmetric climate warming by testing for direct and latent thermal effects on the reproductive ecophysiology of a temperate lizard.

While the effects of environmental temperature on fundamental life processes are apparent in most organisms, they are especially pronounced in ectotherms (reviewed in Angilletta, 2009).

Terrestrial ectotherms are increasingly threatened by recent and predicted declines associated with climate change (Deustch et al., 2008; Pounds, 2001; Sinervo et al., 2010). Some of the more obvious terrestrial ectotherms in an ecosystem are the reptiles, for which the thermal biology is especially well studied, making them ideal model organisms for this research. For example, incubation temperatures in reptiles can affect hatching success and hatchling phenotype (Hare et al., 2002; Shine et al., 1997), as well as subsequent growth (Nelson et al., 2004), survival (Hare et al., 2004), immune response (Les et al., 2009) and reproductive success (Warner et al., 2010). Thus, there are both direct and latent thermal effects associated with the incubation environment of reptiles. Furthermore, daily temperature fluctuations during incubation alone can affect the timing of hatching (Du and Shine, 2010) and hatchling phenotype (Ashmore and Janzen, 2003) beyond the extent observed for constant temperatures, suggesting that the impact of asymmetric warming cannot be discounted. However, the effects of temperature variability on other aspects of reproduction are less clearly understood. In other ecotherms, such as fishes, temperature is partly responsible for seasonal gonadal activity and maturation of oocytes (Koya and Kamiya, 2000). For reptiles in seasonal environments, temperature may act as an exogenous stimulus to alter the largely endogenously controlled rhythm of the ovarian cycle in terms of follicular growth and reproductive timing (Medonça, 1987; Tinkle and Irwin, 1965; Zani and Rollyson, 2011). In at least some viviparous reptiles, both growth rates and phenotypes of hatchlings vary with thermal conditions during the ovarian cycle (Hare and Cree, 2010). However, there is not complete maternal control over embryonic developmental conditions ['maternal manipulation hypothesis' (Shine, 1995)], calling into question the effects of temperature variability on the ovarian cycle. While we know of no reports of temperature during the ovarian cycle of oviparous reptiles affecting subsequent hatchling traits (see also Dubey and Shine, 2011), the existence of latent thermal effects during incubation means that we must consider as a possibility both direct and latent effects of asymmetric warming during the ovarian cycle as well as during incubation.

The thermal dependence of reproductive timing and hatchling traits indicates that ovarian development and incubation are likely to result in life-history changes due to climate warming. While much of the research focus has been on the negative impact of climate change (for a review, see Parmesan, 2006), species currently limited by climate may actually benefit from these life-history changes (Rillig et al., 2002; Chamaillé-Jammes et al., 2006). For example, montane populations of common lizards, *Lacerta vivipara*, have experienced increases in environmental temperatures that have been linked to increases in survival, body size, clutch size and reproductive output (Chamaillé-Jammes et al., 2006). A positive relationship between warmer incubation temperatures and hatchling size has been observed in multiple other lizard species, including both oviparous and viviparous species (Shine, 2004; Shine and Harlow, 1993; Wapstra, 2000; Dubey and Shine, 2011). In general, the effects of climate warming are predicted to increase fitness in temperate ectotherms because of the limitations imposed by current environmental temperatures in seasonal environments (Bradshaw et al., 2004; Deutsch et al., 2008; Huey et al., 2009) (see also Olsson et al., 2010). Thus, we hypothesize that asymmetric warming during the breeding season will increase the fitness of temperate ectotherms by advancing the timing of reproduction, increasing the rate of embryonic development and producing larger hatchlings with a subsequent survival advantage. In order to test these predictions, we simulated different degrees of diurnal temperature variation by manipulating night-time temperature during the breeding season of common side-blotched lizards, *Uta stansburiana* Baird and Girard, near their distributional limits.

MATERIALS AND METHODS
Study organism and study site
Side-blotched lizards are small (40–60 mm adult snout–vent length, SVL) diurnal lizards widely distributed across arid habitats in western North America from Baja California, Mexico north to the Columbia Plateau of Washington state. We collected lizards at Wrights Point (43.437°N, 118.928°W, 1318 m elevation), a large lava outcrop 20 km south of Burns, OR, USA, at the northern edge of the Great Basin Desert. In more southern populations, *Uta* are active year round and females regularly produce multiple clutches of eggs (Cowles, 1941; Sinervo and Doughty, 1996; Tinkle, 1967). The latitude and altitude of this population typically limit *Uta* activity to a short growing season from April to October; females lay one or two clutches of eggs depending on their size/age (Nussbaum et al., 1983; Zani, 2005) and seasonal conditions (Zani and Rollyson, 2011). While mature females regularly lay two clutches (and in some years possibly three), yearlings frequently lay only one clutch unless environmental conditions are especially favorable (Zani, 2005; Zani and Rollyson, 2011).

Ovarian cycle
In order to study the effects of diurnal temperature range for the duration of an ovarian cycle, it was necessary to collect female lizards gravid with their first clutch. Upon oviposition (i.e. day 0 of the second ovarian cycle), temperature could be controlled experimentally for the entirety of the second cycle. We collected gravid females in May–June 2010 and marked each lizard with an identification number by removing a unique combination of three to six toes [never the long (fourth) toe; no more than two toes per foot]. Upon oviposition of a female's first clutch in the lab [for details, see Zani (Zani, 2005)], dam mass (to 0.01 g using an electronic balance) and SVL (to 0.5 mm using a linear rule) were measured. Lizards were then placed into an outdoor common-garden environment of 12 identical 0.8 m² circular cages similar to those described elsewhere (Zani and Rollyson, 2011), but with the addition of mesh covers made from bird block and PVC and a 40×40 cm piece of garden shade cloth for thermal heterogeneity. Cages were set up in an outdoor area ~20 km from the source population and received similar macroenvironmental conditions. Cages were filled to an equal density (three females, one male). Males were provided to maintain a typical social environment and for insemination of second clutches as needed. Males were rotated among cages every 7–10 days to allow females a choice of partners. Lizards were fed crickets, vestigial-wing fruit flies and mealworms *ad libitum*.

Based on the order of ovipostition, each female was assigned to one of three night-time temperature treatments in a systematic fashion (warm then cold then intermediate). However, several females died during the experiment or were excluded for other reasons, so sample sizes of treatment groups were not equal. To inform our choice of temperature manipulation we relied on the most recent summary by the Intergovernmental Panel on Climate Change. The upper range of current climate model predictions is a global average increase of 6.4°C (IPCC, 2007). However, empirical observations indicate that mid- to high-latitudes have experienced greater temperature increases than low latitudes (IPCC, 2007). Pilot

experiments in 2009 (supplementary material Fig. S1) in which we collected temperature data on the natural population indicated that over the course of the breeding season (May–July) the night-time retreats of lizards (rock crevices) transitioned from 17 to 23°C even while the air temperature periodically dropped to near freezing. Thus, for our temperature treatments we used incubators to simulate the possible climatic changes of asymmetric temperature at this site, with 18°C as near natural at the start of the breeding season, 28°C as warmer than natural and near the high range predicted for the 21st century, and 8°C as closer to natural air temperature. This last treatment was chosen because it is possible that lizards behaviorally choose more exposed, cooler retreats and 8°C is near the average night-time low air temperature for the breeding season. However, because this experiment was conducted in a field lab we were somewhat constrained by equipment for our temperature treatments. Thus, our actual treatment temperatures in the night-time incubators were 29±0.1°C (hereafter 'warm'), 17±0.3°C ('intermediate') or 11±0.3°C ('cold') (all measurements are reported as means ± s.e.m.). For comparison of the thermal environments in this study, we recorded temperatures in the natural study area, common-garden (daytime) cages and incubators (night-time). All temperatures were recorded using Watchdog 100-Temp 2K data loggers (Spectrum Technologies, Plainfield, IL, USA).

For logistic reasons, females from one treatment were housed together and each evening around dusk, females (but not males) were removed from cages, placed in cloth bags inside incubators for the night (11.7±0.06 h) and returned to outdoor cages ~2 h after sunrise (~1 h prior to lizard activity). This procedure was repeated nightly from June to July until all females oviposited their second clutch or until it was clear that females were not attempting a second clutch. Abdominal palpations were performed every 5 days to track ovarian cycle progress. We deemed females that failed to progress past the enlarged follicle stage after 30 days of experimental treatment as foregoing a second clutch. Once a female began yolking follicles we searched the outdoor cages daily for signs of nest building. Each female with shelled eggs constructing a nest (i.e. digging) was placed in isolation until oviposition [for details, see Zani (Zani, 2005)], which typically occurred after 2–3 days. To minimize stress during nesting we did not continue exposure to night-time temperature treatment once a female was placed in isolation. On several occasions we were away from the lab overnight, during which time females remained in the outdoor cages and were not exposed to the night-time treatment. No more than four non-consecutive nights were excluded per lizard and all females missed at least two treatment nights during their ovarian cycle. Following completion of this experiment, lizards were released into nearby semi-natural enclosures as per Oregon Department of Fish and Wildlife regulations.

Natural control group

In addition to the second clutches collected from the experimental population, we also collected clutches from females in the natural population known or suspected to be gravid with their second clutch for use as a control. Females in this group remained in the wild until they were determined (by abdominal palpation) to be gravid with shelled eggs, at which point they were brought to the lab to oviposit, which occurred no more than 3 days after collection. Because there is a negative correlation between age and lay date (Zani, 2008; Zani and Rollyson, 2011), determination of first *versus* second clutch was based on age (estimated by SVL) and encounter date. For example, Zani (Zani, 2008) reported that the size *versus* lay date $r=-0.687$ ($P<0.001$).

Incubation

To determine the effects of night-time temperature treatment during incubation, we collected the eggs from second clutches produced by common-garden females. Upon oviposition, all eggs were weighed to within 0.01 g using an electronic balance. Relative clutch mass (RCM) was determined by dividing total clutch mass by dam mass (post-oviposition) to estimate the reproductive effort of females. One fertile egg from each clutch (determined based on appearance) was preserved in 10% formalin and staged following the procedure described elsewhere (Andrews and Greene, 2011) in order to determine the developmental stage and size (length) of embryos at oviposition. The remaining eggs were placed individually in a 75 ml plastic beaker and half-buried on 60 ml of moist substrate (9:1 vermiculite to water by volume), covered with plastic wrap to reduce moisture loss, and placed in sealable 6.7 l plastic cases capable of holding 33 individual eggs. Vermiculite was replaced every 7–10 days. All eggs were incubated on a 12 h:12 h day:night temperature cycle. Daytime incubation conditions were common-garden conditions, in which all eggs were stacked in their boxes in an incubator set to constant warm conditions (29±0.1°C), similar to conditions used previously (Zani, 2008).

Using a split-clutch design, the resulting eggs from dams were systematically (warm, then cold, then intermediate) assigned to one of three night-time temperature incubation treatments based on the laying order of the dams. These treatment environments were identical to those experienced by dams at night during their ovarian cycle, but in this case eggs were assigned individually and each clutch typically had at least one representative egg in each night-time incubation treatment. By reassigning the eggs between ovarian and incubation conditions, we created nine possible night-time temperature environments for individual embryos (3 ovarian environments × 3 incubation environments), which allowed us to separate the thermal effects of ovarian and incubation periods. These nine ovarian:incubation night-time temperature treatments were warm:warm, warm:intermediate, warm:cold, intermediate:warm, intermediate:intermediate, intermediate:cold, cold:warm, cold:intermediate or cold:cold. Thus, each night for 12 h plastic cases containing eggs were transferred to their respective temperature treatment using the same incubators and same set of temperatures for the ovarian temperature treatment (see above). In both daytime and night-time incubators, boxes were rotated daily to minimize incubator effects. Once hatching began, eggs were checked twice daily for hatchlings. The control group was exposed to identical conditions except eggs remained at 29±0.1°C for the duration of incubation.

Hatchlings and release

For all hatchlings, we measured size (mass and SVL) and sex (by presence/absence of enlarged post-anal scales) within 12 h of hatching. Hatchlings were marked (as above) with unique identification numbers and housed for up to 12 days before being shipped overnight to Burns, OR, USA. Hatchlings in the experimental group were released into eight semi-natural field enclosures at a site 24 km north of the source population [for details, see Zani (Zani, 2005; Zani, 2008)]. Briefly, each 100 m² enclosure contains desert habitat, including a portion of 2–4 m high south-facing cliff. Enclosures allow flying insects (i.e. food) to enter while containing lizards within. Each enclosure contains deep cracks in the cliff face as overwintering sites. Aluminium flashing walls were not high enough to prevent most predators (e.g. snakes, birds) from entering (P.A.Z., personal observation). Hatchlings were released into enclosures over 57 days (20 August–15 October 2010) in

randomized (using Microsoft Excel) cohorts containing a mix of treatments at a relative density of $100\,\text{ha}^{-1}$, which is similar to that of the natural population and has no significant impact on growth or survival (Zani, 2008). Hatchlings in the control group were released at the source population. The following spring we used passive pitfall traps (see Zani, 2008) to search enclosures for survivors. Because of inclement weather, trapping occurred from 24 to 27 June 2011 but all survivors were caught on the first day of trapping, which is typical of the enclosures (Zani, 2008). In addition to trapping, enclosures were visually inspected for several hours each day to ensure all animals were recaptured. For the control cohort we daily searched the source population up to 1.5 km from release coordinates to ensure high encounter probability. This distance is ~1.5 times the typical dispersal radius for hatchling *Uta* (Doughty and Sinervo, 1994) (P.A.Z., personal observation). The search for survivors occurred over 19 days from mid-April to mid-July 2011. Survivors were remeasured as above, their reproductive state was noted (females only), and they were released at their site of capture.

Statistical analyses

Statistical analyses were performed using JMP v. 7.0.1 for Macintosh (SAS Institute Inc., Cary, NC, USA). Depending on the type of dependent variable, we conducted regression analyses, one- or two-factor analyses of variance (ANOVA), or one- or two-factor analyses of co-variance (ANCOVA) when relevant covariates could be identified in order to test hypotheses. Because the purpose of these comparisons was to test the variation among treatment groups, for factorial ANCOVA animals from ovarian and/or incubation treatments, but not the control group, were included. For these tests, treatment temperature was included as an independent variable. For each set of ANCOVA we included as covariates dam ID (to take into account possible variation among clutches) and other traits (such as size) thought to affect dependent variables depending on specific comparisons. For example, for analyses of second-clutch characteristics, in addition to dam ID, we sought to include estimates of dam body size and previous reproductive effort as covariates because these were deemed to be of possible influence for second-clutch traits. For body size we used dam mass, but rather than use prior clutch size or clutch mass as covariate, we relied on first clutch RCM (where known), as RCM was originally conceived as an estimate of reproductive effort (Vitt and Congdon, 1978). For all ANCOVA, we first tested for homogeneity of regression coefficients to determine whether ANCOVA was appropriate [see p. 497 of Pedhazur (Pedhazur, 1982)]. To do this we utilized the R^2-change test [see p. 62 of Pedhazur (Pedhazur, 1982)] to calculate the proportion of variance incremented by appropriate product vectors and determined the statistical significance of the resulting F-ratios using Microsoft Excel. Following this we also determined whether each covariate explained significant variation in the dependent variable prior to including that covariate in the final model (using effect tests). Finally, when the above analysis deemed ANCOVA appropriate we tested for differences among treatment groups using effect tests reported by JMP or again using the R^2-change test for each main effect [see p. 501 of Pedhazur (Pedhazur, 1982)]. Because we relied on R^2-change tests to compute ANCOVA, we were able to apply this model to continuous variables as well as categorical traits (e.g. overwintering success). For categorical variables we built a model using the logistic-regression platform in JMP and then used the log-likelihood R^2 (U) for the subsequent R^2-change test. When ANCOVA was deemed inappropriate, we fitted regression equations with separate slopes [see p. 500 of Pedhazur (Pedhazur, 1982)].

When ANCOVA was deemed appropriate but covariates did not explain significant variation, we utilized one- or two-factor ANOVA instead. Where ANOVA indicated significant variation among the treatment groups, we conducted *a posteriori* comparisons between treatment groups using least-squares linear contrasts.

In addition to the above tests, combined treatment groups were analyzed *versus* the control group using a least-squares means comparison to determine whether each combination of ovarian and incubation temperature differed from the control. However, for interclutch interval, an insufficient control sample size prevented us from making this comparison. To test for differences in the sex ratios of hatchlings, we used the Chi-square platform in JMP to compute contingency tables. For these analyses we compared sex ratios of experimental and control animals as well as sex ratios among experimental groups.

RESULTS
Thermal environments

The mean daytime (shaded substrate) temperature for the common-garden environment was 26±0.8°C. The mean daytime (shallow crevice) temperature for the natural population was 29±0.7°C. Rather than compare these means statistically, we conducted a polynomial regression of experimental *versus* natural temperatures between these two environments. This analysis revealed a significant correlation ($F_{2,30}$=98.28, P<0.001, R^2=0.868) with both linear ($F_{1,30}$=141.34, P<0.001) and non-linear (quadratic: $F_{1,30}$=24.62, P<0.001) components to temperature variation. These results suggest that changes in the natural environment were paralleled by the daytime experimental environment.

The mean night-time (shallow crevice) temperature from the natural population was 22±0.4°C. However, as we noted above, this increased during the course of the spring. ANOVA comparing the natural night-time temperatures with each experimental treatment indicated that the warm treatment had a significantly higher (+7°C) average night-time temperature than natural ($F_{1,192}$=224.25, P<0.001), and both the intermediate and cold treatments had significantly lower (–4 and –11°C, respectively) average night-time temperatures than natural ($F_{1,209}$=85.19, P<0.001 and $F_{1,209}$=574.37, P<0.001, respectively). As expected, natural variation in night-time temperature was between the warm and intermediate experimental night-time environments.

Second-clutch characteristics

Fifty-nine females laid their first clutch in the lab and were included in our experimental population. Of these, 36 (61.0%) successfully laid second clutches, yielding 141 fertile eggs; 15 of 19 (78.9%) from the warm treatment, 12 of 17 (70.6%) from the intermediate treatment, and 9 of 23 (39.1%) from the cold treatment. As successful completion of a second clutch in nature is related to dam size and possibly to previous reproductive effort, we included dam ID, dam mass and first clutch RCM as covariates when testing to determine whether ovarian temperatures influenced the probability of laying a second clutch. Dam ID did not explain significant variation in the probability of laying a second clutch, but both dam mass and RCM did and were retained in the analysis. We then tested for heterogeneity of regression coefficients (a pre-requisite of ANCOVA) and found no variation among treatment levels, indicating ANCOVA was appropriate. Next, we detected significant variation among treatment intercepts ($F_{2,54}$=4.89, P=0.011), which indicated a positive relationship between night-time treatment temperature and laying of a second clutch. That is, even after the effects of body size and previous reproductive effort of females were
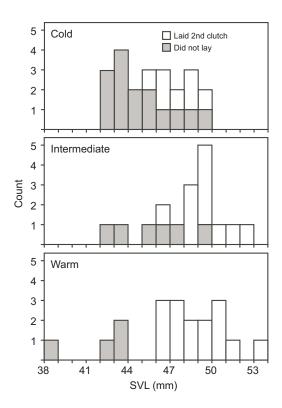
Fig. 1. Histograms showing second clutch success (laid, did not lay) *versus* body size (snout–vent length, SVL) for females exposed to one of three night-time temperature treatments.

taken into account, warmer night-time temperatures doubled the probability that females would lay a second clutch compared with those in the cold treatment (78.9% successful second clutches *versus* 39.1%, respectively; Fig. 1).

We collected 21 additional females from the natural population prior to laying their second clutch, which acted as our control group, and compared characteristics of second clutches of the control with each experimental group separately including dam ID and dam mass as covariates. When we tested for heterogeneity of regression coefficients among the experimental treatments we found no significant variation for any treatment in comparison to the control. In addition, dam ID did not explain variation in any second-clutch trait and was excluded, while dam mass was only retained for analysis of lay date. Tests for heterogeneity of intercepts among lay dates revealed that the warm treatment group laid on average 1.8 days later than the control, which is not significantly different ($F_{1,34}=3.35$, $P=0.076$; Table 1), but there was a significantly delayed lay date for the intermediate ($F_{1,31}=16.72$, $P<0.001$) and cold ($F_{1,28}=11.17$,

$P=0.002$) treatment groups of 7.3 and 9.5 days, respectively. For both developmental stage and length of embryos at oviposition we found no difference between the control and any of the treatment groups ($P>0.240$). For all of the remaining second-clutch characteristics there was no significant covariate and subsequent analyses were conducted as one-factor ANOVA with ovarian temperature treatment as the main effect. The mean size of second clutches of the control was different (1.0 eggs larger) from that of the cold treatment only ($F_{1,28}=7.00$, $P=0.013$; Table 1); neither warm nor intermediate second-clutch sizes differed from the control ($F_{1,34}=3.99$, $P=0.054$ and $F_{1,31}=1.16$, $P=0.291$, respectively). For all treatment groups, average egg mass of second clutches was greater than that of the control (warm: $F_{1,34}=21.05$, $P<0.001$; intermediate: $F_{1,31}=15.07$, $P<0.001$; cold: $F_{1,28}=4.51$, $P=0.043$; Table 1). However, RCM of females was no different between control and experimental treatments (warm: $F_{1,34}=1.00$, $P=0.324$; intermediate: $F_{1,31}=0.26$, $P=0.611$; cold: $F_{1,28}=0.51$, $P=0.482$; Table 1) suggesting that the reproductive effort of dams was not affected by the experiment.

Following comparison with the control group, we examined second-clutch characteristics among the treatment groups for heterogeneity of regression coefficients using dam ID, dam mass and first clutch RCM as covariates. However, for all dependent variables no covariate explained significant variation and these were therefore removed from analyses. Subsequent analyses on second-clutch characteristics (Table 1) were conducted as one-factor ANOVA with ovarian temperature treatment as main effect. Clutch size ($F_{2,31}=0.72$, $P=0.490$), mean egg mass ($F_{2,31}=0.46$, $P=0.635$), RCM ($F_{2,31}=0.01$, $P=0.906$), embryo stage ($F_{2,29}=0.46$, $P=0.636$) and embryo size ($F_{2,29}=0.26$, $P=0.770$) (supplementary material Fig. S2) were not related to experimental temperature. However, interclutch interval was negatively related to night-time temperature ($F_{2,31}=12.82$, $P<0.001$; Fig. 2, Table 1). *A posteriori* least-squares means contrasts comparing the interclutch interval indicated significant differences between the warm treatment and both intermediate ($F_{1,31}=13.27$, $P<0.001$) and cold ($F_{1,31}=21.58$, $P<0.001$) groups, but not between intermediate and cold treatments ($F_{1,31}=1.60$, $P=0.215$).

Incubation and hatchlings

To test for immediate and latent effects of temperature on incubation period, we included as factors both ovarian and incubation temperature and their interaction in a factorial ANCOVA in which dam ID, dam mass and initial egg mass were included as covariates. While we detected no heterogeneity among regression coefficients, neither dam ID nor dam mass explained significant variation and were removed as covariates. The resulting ANCOVA model (with initial egg mass as covariate) revealed that both ovarian ($F_{2,58}=6.82$, $P=0.002$) and incubation temperature ($F_{2,58}=1140.13$, $P<0.001$; Table 2), but not their interaction ($F_{4,58}=0.74$, $P=0.568$), affected the length of the incubation period. The magnitude of F-ratios from

Table 1. Mean ± s.e.m. clutch and embryo characteristics for second clutches of natural (control) and experimental cohorts

Ovarian treatment	N	Lay date (Julian day)	Clutch size	Egg mass (g)	Relative clutch mass[1]	Interclutch interval (days)[2]	Embryo stage	Embryo length (mm)
Control	21	195.0±1.37	4.8±0.18	0.27±0.008	0.48±0.019	31.0±4.32	27.9±0.28	2.2±0.09
Warm	15	196.8±2.75[n.s.]	4.1±0.37[n.s.]	0.32±0.009***	0.43±0.042[n.s.]	26.5±1.39	27.3±0.25[n.s.]	2.1±0.08[n.s.]
Intermediate	12	202.3±2.33*	4.5±0.31[n.s.]	0.32±0.011***	0.50±0.039[n.s.]	32.9±1.08	27.0±0.45[n.s.]	2.1±0.11[n.s.]
Cold	9	204.5±2.75**	3.8±0.45*	0.31±0.021*	0.44±0.057[n.s.]	35.5±1.09	27.5±0.42[n.s.]	2.2±0.18[n.s.]

[1]RCM, calculated as clutch mass divided by dam mass.
[2]Insufficient sample size in control for comparison.
Asterisks indicate statistical significance between each treatment and control (*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$; n.s., not significant).
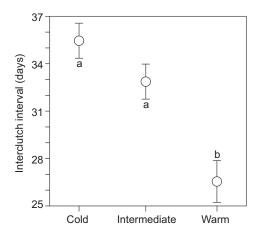
Fig. 2. Mean duration of interclutch interval (±s.e.m.) for females exposed to one of three night-time temperature treatments during the ovarian cycle. Letters associated with each group indicate statistical significance within that experimental treatment; groups with letters in common are not significantly different whereas different letters indicate a statistical difference ($P<0.05$).

these analyses suggests that while there are both direct and latent effects, incubation length is primarily determined by the immediate incubation environment.

Of 141 fertile eggs in the experimental treatments, we preserved 36 for staging and incubated the remaining 105 eggs. Of these 105 eggs, 67 (63.8%) hatched between 40 and 90 days. Of the 84 eggs obtained for the control, 45 (53.6%) hatched, but with a much narrower range of incubation (39–44 days). An analysis between all experimental groups and the control again with dam ID, dam mass and initial egg mass as covariates indictated no heterogeneity among regression coefficients, but neither dam ID nor dam mass explained significant variation in hatching success. The resulting ANCOVA model (with initial egg mass as covariate) revealed that experimental animals had significantly higher incubation success than the control ($F_{1,148}=40.24$, $P<0.001$). However, as incubation temperature is related to hatching success in other species, we conducted an additional analysis on experimental animals in which both ovarian and incubation treatments and their interaction were included as factors and dam ID, dam mass and egg mass were included as covariates. Again, homogeneity of regression coefficients indicated ANCOVA was appropriate, but neither dam ID nor dam mass was a significant covariate and they were removed from analyses. Despite

the differences in incubation period (Table 2), the resulting model revealed no significant survival effects due to ovarian ($F_{2,96}=0.92$, $P=0.404$) or incubation temperature ($F_{2,96}=1.21$, $P=0.303$) or their interaction ($F_{4,96}=0.65$, $P=0.629$).

A two-by-two contingency table indicated that the sex ratio of hatchlings in the control was not different from that of all experimental hatchlings combined ($\chi^2=2.28$, $P=0.131$; Table 3). As the control and warm incubation treatment differed only in the pre-incubation conditions experienced by the embryos, as a further test of experimental conditions we compared the sex ratio of the control with the warm incubation treatment. A two-by-two contingency table indicated no difference in hatchling sex ratio ($\chi^2=0.37$, $P=0.542$; Table 3). Following this we compared sex ratios among experimental treatments alone. Neither ovarian temperature treatment ($\chi^2=0.325$, $P=0.850$) nor incubation temperature treatment ($\chi^2=5.77$, $P=0.056$) was related to hatchling sex ratio (Table 3).

We found no difference in hatchling size between males and females (mass: $F_{1,65}=0.55$, $P=0.460$; SVL: $F_{1,65}=2.48$, $P=0.120$) and therefore did not include hatchling sex in subsequent analyses. However, initial egg mass was positively related to both hatchling mass ($F_{1,65}=68.82$, $P<0.001$) and SVL ($F_{1,65}=35.07$, $P<0.001$). Thus, we again included dam ID and initial egg mass as covariates in an analysis in which ovarian and incubation temperature treatments and their interaction were included as factors to explain variation in hatchling size. However, dam ID was not a significant predictor of hatchling size and was removed from analyses. For hatchling mass, ANCOVA indicated a significant effect of incubation temperature treatment ($F_{2,58}=7.32$, $P=0.001$; Fig. 3), but no effect of ovarian temperature ($F_{2,58}=0.09$, $P=0.910$) or their interaction ($F_{4,58}=1.46$, $P=0.226$). Thus, there appear to be direct thermal effects due to incubation, but no latent effects on hatchling phenotype due to ovarian environment. For hatchling SVL, heterogeneity of variance indicated ANCOVA was not appropriate ($F_{4,54}=14.70$, $P<0.001$). Instead, we determined separately the relationship between initial egg mass and hatchling SVL for each level of ovarian (warm: $Y=11.2b+19.2$, $F_{1,30}=8.02$, $P=0.008$; intermediate: $Y=14.0b+18.7$, $F_{1,19}=4.30$, $P=0.052$; cold: $Y=25.4b+14.6$, $F_{1,13}=9.96$, $P=0.008$) and incubation treatments (warm: $Y=12.8b+19.4$, $F_{1,19}=24.68$, $P<0.001$; intermediate: $Y=17.8b+17.3$, $F_{1,22}=1.07$, $P=0.009$; cold: $Y=4.7b+20.8$, $F_{1,21}=0.65$, $P=0.430$). These findings indicate that heavier eggs resulted in longer hatchlings in animals from the cold ovarian treatment and both the warm and intermediate incubation treatments.

To test for the effects of temperature during the entire developmental period (i.e. interclutch interval and incubation period

Table 2. Mean ± s.e.m. hatchling characteristics for second clutches of natural (control) and experimental cohorts

Ovarian treatment	Incubation treatment	Sample size (N)	Egg mass (g)	Incubation period (days)	Hatchling mass (g)	Hatchling SVL (mm)	Embryonic period[1]
Control	Control	44	0.29±0.005	40.5±0.18	0.41±0.006	22.67±0.099	N/A
Warm	Warm	18	0.33±0.010***	41.6±0.45[n.s.]	0.45±0.016[n.s.]	23.41±0.237[n.s.]	68.2±1.72
	Intermediate	13	0.32±0.010*	69.8±1.25***	0.41±0.015*	22.68±0.305[n.s.]	96.7±1.46
	Cold	16	0.32±0.009*	83.2±0.87***	0.40±0.018*	22.33±0.163***	110.3±1.97
Intermediate	Warm	11	0.32±0.011*	43.0±0.37***	0.46±0.021[n.s.]	23.83±0.211**	75.7±1.33
	Intermediate	14	0.31±0.009*	73.9±0.69***	0.43±0.023[n.s.]	23.56±0.286*	107.5±1.21
	Cold	13	0.31±0.007*	86.3±1.34***	0.37±0.020***	22.11±0.381**	119.1±2.30
Cold	Warm	8	0.31±0.021[n.s.]	41.7±0.33[n.s.]	0.48±0.045[n.s.]	23.92±0.507[n.s.]	76.7±2.96
	Intermediate	8	0.31±0.022[n.s.]	71.8±1.30***	0.43±0.039[n.s.]	22.71±0.881[n.s.]	106.7±1.54
	Cold	6	0.30±0.020[n.s.]	83.0±1.30***	0.41±0.023[n.s.]	22.60±0.203[n.s.]	117.6±1.69

[1]Insufficient sample size of control interclutch interval for comparison.

SVL, snout–vent length.

Asterisks indicate statistical significance between each treatment and control (*$P\le0.05$, **$P\le0.01$, ***$P\le0.001$; n.s., not significant).

Table 3. Number (and %) of male and female hatchlings for second clutches of natural (control) and experimental cohorts

Treatment	Male:female (%)
Control	28:17 (62.2:37.8)
Ovarian	
Warm	15:17 (46.9:53.1)
Intermediate	11:10 (52.4:47.6)
Cold	6:8 (42.9:57.1)
Incubation	
Warm	14:6 (70.0:30.0)
Intermediate	9:15 (37.5:62.5)
Cold	9:14 (39.1:60.9)

combined), we included both ovarian and incubation temperature and their interaction in a factorial ANCOVA in which dam ID, dam mass and initial egg mass were included as covariates. While we detected no heterogeneity among regression coefficients, no covariate explained significant variation in embryonic period and they were removed. The resulting two-factor ANOVA revealed that both ovarian ($F_{2,59}$=27.93, P<0.001; Fig. 4) and incubation temperature ($F_{2,59}$=114.05, P<0.001; Table 2), but not their interaction ($F_{4,59}$=0.28, P=0.888), affected embryonic period. Again, the magnitude of F-ratios suggests that incubation environment is relatively more important than ovarian environment in determining the overall length of the embryonic period.

Overwinter survival

Of the 67 experimental hatchlings, only five (7.5%) survived the winter in enclosures (Fig. 5). To test the effects of experimental treatment on overwinter survival, we included both ovarian and incubation treatments and their interaction as factors in a logistic regression with dam ID, initial hatchling mass and hatch date as covariates. However, no covariate explained significant variation and they were removed. In the resulting two-factor logistic regression, variation in winter survival was explained by incubation temperature (d.f.=2, χ^2=7.81, P=0.020), but not ovarian temperature (d.f.=2, χ^2<0.01, P=0.999) or their interaction (d.f.=4, χ^2=1.98, P=0.739). As both experimental treatments significantly affected

the timing of hatching, we replaced both factors and their interaction with hatch date as an independent variable to predict survival and found a significant relationship (Julian date, JD for 'yes'=243.4±6.22; 'no'=266.0±2.34; d.f.=1, χ^2=6.59, P=0.010; Fig. 5) with no survivor hatching later than JD=267 (24 September). Thus, the timing of hatching, driven primarily by incubation temperature, appears to determine overwinter survival of hatchlings.

Of the 45 control hatchlings, six (13.3%) survived the winter in the natural environment (Fig. 5). For the control, we tested whether hatching date affected survival in nature with dam ID, initial hatchling mass and lay date as covariates. However, no covariate explained significant variation and they were excluded. Logistic regression with hatch date alone was unable to explain variation in winter survival (d.f.=1, χ^2=0.10, P=0.749; Fig. 5). While survivors (JD=232.5±0.50) and non-survivors (JD=233.2±0.94) differed by only 1 day in mean hatch date, both groups hatched on average over 10 days earlier than experimental animals, suggesting that the effects of hatch date were only detectable by experimentally extending hatching beyond the natural range of variation.

DISCUSSION

Climate models [for summary, see IPCC data (IPCC, 2007)] predict global average temperatures to rise by as much as 6.4°C by the end of the century. Empirical observations have suggested that much of the recent warming is diurnally asymmetric and has occurred primarily by an increase in night-time temperatures (DeGaetano and Allen, 2002; Easterling et al., 1997; Easterling et al., 2000; Karl et al., 1991; Karl et al., 1993) especially during winter (Easterling et al., 1997). While we know of no predictions that explicitly suggest future warming will occur primarily at night, it is clear from the results of this study that in addition to dam body size, night-time temperature during the ovarian cycle affects the probability (Fig. 1) and timing (Fig. 2) of laying a second clutch. Thus, higher temperatures, even if they only occur at night, are capable of causing an increase in annual reproductive output independent of growing season length. Although other studies have reported decreases in reproductive output above optimal temperatures due to physiologic stress (Luo et al., 2010), asymmetric climate warming could increase mean environmental temperatures without surpassing a species'
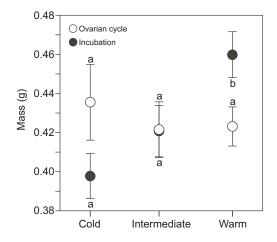

Fig. 3. Mean mass (±s.e.m.) of hatchlings after exposure to one of three night-time temperature treatments either in the ovarian cycle or during incubation. Letters associated with each group indicate statistical significance within that experimental treatment; groups with letters in common are not significantly different whereas different letters indicate a statistical difference (P<0.05).
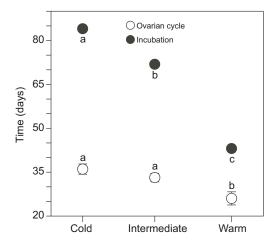

Fig. 4. Mean duration (±s.e.m.) of ovarian cycle or incubation of embryos during exposure to one of three night-time temperature treatments. Letters associated with each group indicate statistical significance within that experimental treatment; groups with letters in common are not significantly different whereas different letters indicate a statistical difference (P<0.05).
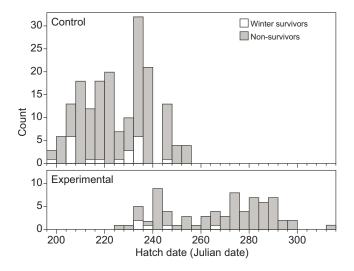
Fig. 5. Histograms showing timing of hatching (as Julian date, days since 1 January) for control and experimental lizards. For the control group, all hatchlings from 2010 are included, not just those from second clutches; statistical analysis was conducted on second clutches only. Overwinter survival or mortality of each hatchling is also indicated.

thermal maximum as temperatures in the lower portion of the distribution rise disproportionately. Our data suggest that this would allow for an overall increase in clutch frequency and possibly subsequent survival among temperate ectotherms, a pattern reported previously (Bradshaw et al., 2004). Two major factors comprising the evolutionary fitness of dams are reproduction and survival, and clearly a doubling of reproductive output by successful second clutches may increase immediate fitness in the face of climate warming. Furthermore, our results indicate that the length of the ovarian cycle is negatively related to temperature treatment (Fig. 2), which may not only increase reproductive frequency of individuals but also minimize mortality risk during reproduction. For example, the locomotor capacity of female *Uta* and other lizards is reduced while gravid (Zani et al., 2008), which could reduce escape ability and represent a reproductive cost (*sensu* Shine, 1980). Shortening this vulnerable period in the female life cycle by speeding up the ovarian cycle could increase female survival, again increasing fitness. Thus, a major finding of this study is that climate change is likely to produce fitness benefits at multiple levels of integration for temperate ectotherms.

Not only does it appear that climate warming will benefit dams but also there are potential fitness benefits for the hatchlings of temperate ectotherms as a result of thermal effects during incubation. We found that hatchling mass was positively correlated with incubation temperature, as noted previously (Dubey and Shine, 2011), but not significantly affected by ovarian temperature (Fig. 3). By what mechanism does incubation temperature influence hatchling traits? Previous studies of skinks (*Bassiana duperreyi*) showed that embryonic heart rate increases exponentially with temperature during incubation (Du and Shine, 2010). Such a thermal effect in the present study could explain the apparent non-linear temperature dependence of development during both the ovarian (Fig. 2) and incubation (Fig. 4) periods. Oufiero and Angilletta (Oufiero and Angilletta, 2010) recently demonstrated in eastern fence lizards (*Sceloporus undulatus*) that incubation at fluctuating temperatures alters the rate and duration of embryonic development but not the energetic cost. Thus, temperature appears to alter the rate of

embryonic development by changing factors such as embryonic heart rate, but not developmental costs or the embryonic stage at oviposition (Table 1). This presents one possible explanation for the observed differences in hatchling size between temperature treatments: embryos developing at colder temperatures may utilize equal yolk reserves for basic metabolic processes as warm embryos, but they do so for a substantially longer period of time (up to twice as long) because of the slower rate of embryonic development, resulting in a smaller amount of yolk being allocated to growth.

The results of our experiment indicate that there are direct thermal effects on hatchling phenotype from the incubation environment, but not latent effects from the temperature exposure of the dam during the ovarian cycle. While we did not detect latent effects on hatchling phenotypes, we did find evidence that temperature during the ovarian cycle affects subsequent incubation; namely, the length of incubation is primarily due to direct effects of the incubation environment (Fig. 4), yet a small, but significant, portion of the incubation period can be explained by ovarian temperature. While suggestive, the absence of a clear latent thermal effect is similar to that reported for other ectotherm species. For example, larval pitcher-plant mosquitoes (*Wyeomyia smithii*) showed immediate survival reduction when exposed to thermal stress, but no reduction in subsequent adult reproductive output (Zani et al., 2005). However, these direct thermal effects on survival were enough to reduce cohort fitness, measured as *per capita* offspring produced (R_o), to the point where some populations were imperiled (Zani et al., 2005).

We found that many aspects of lizard reproduction were temperature independent. Thus clutch size, egg mass, RCM, and embryo stage and size at oviposition did not vary among females in the different ovarian temperature treatments. However, we did detect differences in clutch size when comparing the experimental and control populations, such that the clutch size of experimental treatment groups tended to be smaller (0.4–1.1 fewer eggs per clutch) than second clutches from the natural population (Table 1). In addition, mean egg mass in experimental treatments was greater (0.04–0.05 g heavier eggs) when compared with that of the natural population. While this might indicate a difference in maternal investment, as there was no difference in RCM between experimental and control groups (Table 1), we interpret these results as merely differences in allocation strategy (i.e. egg size *versus* number) possibly induced by experimental conditions (but see Luo et al., 2010). Another explanation for this result is that some factor, such as water content and hence egg mass, differed, but not maternal investment in yolk. Water content is a function of clutch size relative to female size (Qualls and Andrews, 1999). Therefore, increased water availability in the experimental environment might result in increased egg mass independent of maternal investment. We should note that we watered potted sagebrush in each common-garden cage every 3–4 days while the same period was fairly dry at the source population. Although the experimental environment was designed to mimic the natural environment, the availability of water and food was likely higher in the common-garden environment. Previous research in lizards has shown that relatively low food availability reduces both clutch size and number (Ballinger, 1977). Furthermore, high population density in *Uta* reduces the clutch size of females through stress-induced hormonal pathways (Sinervo et al., 2000). In addition to the relatively high local density in the common-garden environment (four lizards in 0.8 m^2), manipulations, such as daily handling, palpating or housing in an unfamiliar enclosure, are known to increase plasma corticosterone in lizards (Langkilde and Shine, 2006). Corticosterone, in turn, has been linked to reproductive physiology in this species (for a review,

see Sinervo and Calsbeek, 2003). As animals were housed in an enclosure for the duration of their second clutch, handled daily when exposed to night-time treatments, palpated every 5 days to score gravidity, and placed in isolation chambers for oviposition, it is highly probable that the differences in clutch size and egg mass we observed were in some way related to experimental conditions. Unfortunately, because a sham control group that received equal handling but no overnight temperature treatment was not used in this study, it is impossible to say whether clutch characteristics were affected by handling alone. Thus, whether temperature differences between experimental and control groups also affected these life-history traits cannot be determined, but as animals experienced a common-garden environment except for night-time temperature treatments, the primary statistical comparison this may confound is between experimental and control populations and not among levels of experimental treatment.

As noted above, we detected a relationship between incubation temperature and hatchling phenotype in this study; warmer incubation temperatures produced heavier, longer hatchlings. This type of direct effect of temperature on hatchling phenotype in reptiles has been documented numerous times (for a review, see Booth, 2006) and is not limited to body size; temperature also affects shape, color, behavior and growth. Hatchling size, in turn, affects fitness in *Uta* (Ferguson and Fox, 1984), as well as in other lizards (Andrews et al., 2000; Telemeco et al., 2010), suggesting that climate warming could increase lizard fitness as a result of changes in offspring size alone. Incubation temperature also affects hatchling thermoregulation (Goodman and Walguarnery, 2007) and locomotor performance (Elphick and Shine, 1998; Hare et al., 2008), which could impact fitness. A complicating factor for determining potential thermal effects on fitness is that any benefit from increased incubation temperature in nature is dependent upon dam nest-site choice, which has been shown to affect both nest temperature and resulting hatchling phenotype (Doody et al., 2006; Schwanz and Janzen, 2008; Weisrock and Janzen, 1999). For example, Doody and colleagues (Doody et al., 2006) showed that female water dragons (*Physignathus lesueurii*) in Australia compensated for thermal differences among populations along a latitudinal cline by choosing nest sites with appropriate thermal characteristics. Thus, one caveat to the potential for climate change to impact thermal environments during incubation is that females have the ability to mediate spatial or temporal changes to the thermal environment of nests. A second caveat is that as observed changes in diurnal temperature range are smallest in summer (Easterling et al., 1997), temperatures may not be increasing substantially at a time (summer) when eggs are actually incubating. Thus, despite the fact that lizard incubation is temperature dependent, it is not yet clear that climate change at this site results in thermal effects during the incubation period or that females will be unable to mitigate these effects *via* nest-site choice. In other words, the potential fitness costs or benefits identified in experiments such as ours may or may not be realized by animals in the natural environment and should be considered 'potential' effects in the absence of empirical evidence. Still, all indications are that warming incubation environments due to climate change could benefit temperate ectotherms by producing larger, more robust hatchlings with a greater ability to survive. Contrary to expectations, we did not detect a size advantage in overwinter survival of hatchlings; larger hatchlings appeared no more or less likely to survive the winter. However, this may have been due to stochastic variation in survival associated with the relatively small sample size of hatchlings (*N*=67 experimental, 45 control). Yet, we did detect a timing effect in the experimental group; earlier

hatchlings were more likely survive (Fig. 5). Even if hatchling traits are unaffected by climate change, there is likely a fitness benefit from the advancement of hatching date and this may obscure any size advantage.

We found that the total embryonic period (from laying of the first clutch to hatching of the second) is negatively correlated to temperature during both the ovarian cycle and incubation, although incubation temperature appears to have a more pronounced effect (Fig. 4). A shorter embryonic period would result in advanced phenology (earlier laying and hatching), which in turn would allow hatchlings more time to grow and store energy for winter. Consistent with this, we found that overwinter survival was significantly higher for lizards that hatched earlier, though again we note the relatively small sample size involved. As our results demonstrate, altered night-time temperatures can cause significant lengthening of the embryonic period (cold treatment was 155% longer than warm; Table 2). Developing embryos do not appear to have the capacity to compensate physiologically for cold temperatures during incubation (Booth, 1998) (but see Du et al., 2011) or to hatch prematurely in response to decreasing temperatures, such as those experienced in autumn (Shine, 2002). Advancing reproductive phenology is one of the most likely means of maximizing fitness in seasonal environments, and is a common pattern in many lizards (Sinervo and Doughty, 1996; Warner and Shine, 2007) as well as other species, such as fishes (Schultz, 1993), insects (Landa, 1992) and birds (Verhulst, 1998). To this end, by delaying hatching in our experimental cohorts beyond that of natural variation we were able to show that overwinter survival is related to hatching phenology. In particular, it appears that hatchlings need about 6 weeks to grow and/or store sufficient energy to survive the winters of eastern Oregon; no lizard hatching within 6 weeks of the end of the growing season survived in this experiment. This finding is consistent with our experimental observations that larger individuals from this same population survive winter for longer periods, possibly due to energetic constraints (Zani, 2008), and that individuals <1 g at the onset of winter rarely survive in nature or the lab (P.A.Z., personal observation).

It is beyond the scope of this study to predict the impact of the fitness changes we detected. However, the present study is one of the first to verify experimentally the responses of temperate ectotherms to ongoing climate change (see also Chamaillé-Jammes et al., 2006; Huey et al., 2009; Olsson et al., 2010), and to test the effects of asymmetric changes to diurnal temperature range. Based on our results, we conclude that these effects are primarily beneficial for this population of northern lizards, and predict that future changes will continue to benefit the reproduction, growth and survival of individuals at this site. Temperatures are rising more rapidly at mid- to high-latitudes (IPCC, 2007), and organisms living at higher latitudes appear to be affected disproportionately by climate change (Parmesan, 2006; Parmesan, 2007; Root et al., 2003). However, recent predictions suggest that tropical species of terrestrial ectotherms may also be severely impacted (Deutsch et al., 2008). Indeed, extinctions among reptiles have begun to be reported at low latitudes (Sinervo et al., 2010). For this reason, increasing our ability to understand and predict biological responses to climate change is of crucial importance.

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REFERENCES

Andrews, R. M. and Greene, V. W. (2011). Character variation in embryonic development of the lizard *Uta stansburiana*. *J. Herpetol.* **45**, 451-454.

Andrews, R. M., Mathies, T. and Warner, D. A. (2000). Effect of incubation temperature on morphology, growth, and survival of juvenile *Sceloporus undulatus*. *Herp. Monogr.* **14**, 420-431.

Angilletta, M. J., Jr (2009). *Thermal Adaptation: a Theoretical and Empirical Synthesis.* Oxford: Oxford University Press.

Ashmore, G. M. and Janzen, F. J. (2003). Phenotypic variation in smooth softshell turtles (*Apalone mutica*) from eggs incubated in constant versus fluctuating temperatures. *Oecologia* **142**, 182-188.

Ballinger, R. E. (1977). Reproductive strategies: food availability as a source of proximal variation in a lizard. *Ecology* **58**, 628-635.

Booth, D. T. (1998). Incubation of turtle eggs at different temperatures: do embryos compensate for temperature during development? *Physiol. Zool.* **71**, 23-26.

Booth, D. T. (2006). Influence of incubation temperature on hatchling phenotype in reptiles. *Physiol. Biochem. Zool.* **79**, 274-281.

Bradshaw, W. E. and Holzapfel, C. M. (2006). Evolutionary response to rapid climate change. *Science* **312**, 1477-1478.

Bradshaw, W. E., Zani, P. A. and Holzapfel, C. M. (2004). Adaptation to temperate climates. *Evolution* **58**, 1748-1762.

Buckley, L. B. (2010). The range implications of lizard traits in changing environments. *Global Ecol. Biogeogr.* **19**, 452-464.

Chamaillé-Jammes, S., Massot, M., Aragón, P. and Clobert, J. (2006). Global warming and positive fitness response in mountain populations of common lizards *Lacerta vivipara*. *Global Change Biol.* **12**, 392-402.

Constable, J. V. and Retzlaff, W. A. (2000). Asymmetric day/night temperature elevation: growth implications for yellow poplar and loblolly pine using simulation modeling. *Forest Sci.* **46**, 248-257.

Cowles, R. B. (1941). Observations on the winter activities of desert reptiles. *Ecology* **22**, 125-140.

DeGaetano, A. T. and Allen, R. J. (2002). Trends in twentieth-century temperature extremes across the United States. *J. Clim.* **15**, 3188-3205.

Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C. and Martin, P. R. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proc. Natl. Acad. Sci. USA* **105**, 6668-6672.

Doody, J. S., Guarino, E., Georges, A., Corey, B., Murray, G. and Ewert, M. (2006). Nest site choice compensate for climate effects on sex ratios in a lizard with environmental sex determination. *Evol. Ecol.* **20**, 307-330.

Doughty, P. and Sinervo, B. (1994). The effects of habitat, time of hatching, and body size on the dispersal of hatchling *Uta stansburiana*. *J. Herpetol.* **28**, 485-490.

Du, W. G. and Shine, R. (2010). Why do the eggs of lizards (*Bassiana duperreyi*: Scincidae) hatch sooner if incubated at fluctuating rather than constant temperatures? *Biol. J. Linn. Soc.* **101**, 642-650.

Du, W. G., Zhao, B., Chen, Y. and Shine, R. (2011). Behavioral thermoregulation by turtle embryos. *Proc. Natl. Acad. Sci. USA* **108**, 9513-9515.

Dubey, S. and Shine, R. (2011). Predicting the effects of climate change on reproductive fitness of an endangered montane lizard, *Eulamprus leuraensis* (Scincidae). *Clim. Change* **107**, 531-547.

Easterling, D. R., Horton, B., Jones, P. D., Peterson, T. C., Karl, T. R., Parker, D. E., Salinger, M. J., Razuvayev, V., Plummer, N., Jamason, P. et al. (1997). Maximum and minimum temperature trends for the globe. *Science* **277**, 364-367.

Easterling, D. R., Karl, T. R., Gallo, K. P., Robinson, D. A., Trenberth, K. E. and Dai, A. (2000). Observed climate variability and change of relevance to the biosphere. *J. Geophys. Res.* **105**, 20101-20114.

Elphick, M. J. and Shine, R. (1998). Longterm effects of incubation temperature on the morphology and locomotor performance of hatchling lizards (*Bassiana duperreyi*, Scincidae). *Biol. J. Linn. Soc.* **63**, 429-447.

Ferguson, G. W. and Fox, S. F. (1984). Annual variation of survival advantage of large juvenile side-blotched lizards, *Uta stansburiana*: its causes and evolutionary significance. *Evolution* **38**, 342-349.

Goodman, R. M. and Walguarnery, J. W. (2007). Incubation temperature modifies neonatal thermoregulation in the lizard *Anolis carolinensis*. *J. Exp. Zool.* **307**, 439-448.

Hare, K. M. and Cree, A. (2010). Exploring the consequences of climate-induced changes in cloud cover on offspring of a cool-temperate viviparous lizard. *Biol. J. Linn. Soc.* **101**, 844-851.

Hare, K. M., Daugherty, C. H. and Cree, A. (2002). Incubation regime affects juvenile morphology and hatching success, but not sex, of the oviparous lizard *Oligosoma suteri*. *New Zeal. J. Zool.* **29**, 221-229.

Hare, K. M., Longson, C. G., Pledger, S. and Daugherty, C. H. (2004). Size, growth, and survival are reduced at cool incubation temperatures in the temperate lizard *Oligosoma suteri*. *Copeia* **2004**, 383-390.

Hare, K. M., Pledger, S. and Daugherty, C. H. (2008). Low incubation temperatures negatively influence locomotor performance and behavior of the nocturnal lizard *Oligosoma suteri*. *Copeia* **2008**, 16-22.

Huey, R. B., Deutsch, C. A., Tewksbury, J. J., Vitt, L. J., Hertz, P. E., Álvarez Pérez, H. J. and Garland, T., Jr (2009). Why tropical forest lizards are vulnerable to climate warming. *Proc. Biol. Sci.* **276**, 1939-1948.

IPCC (2007). *Climate Change 2007: Impacts, Adaptation, and Vulnerability.* Cambridge: Cambridge University Press.

Karl, T. R., Kukla, G., Razuvayev, V. N., Changery, M. J., Quayle, R. G., Heim, R. R., Jr, Easterling, D. R. and Fu, C. B. (1991). Global warming: evidence for asymmetric diurnal temperature change. *Geophys. Res. Lett.* **18**, 2253-2256.

Karl, T. R., Jones, P. D., Knight, R. W., Kukla, G., Plummer, N., Razuvayev, V., Gallo, K. P., Lindsay, J., Charlson, P. J. and Peterson, T. C. (1993). Asymmetric trends of daily maximum and minimum temperature. *Bull. Am. Meteor. Soc.* **74**, 1007-1023.

Koya, Y. and Kamiya, E. (2000). Environmental regulation of annual reproductive cycle in the mosquitofish, *Gambusia affinis*. *J. Exp. Zool.* **286**, 204-211.

Lambrechts, L., Paaijmans, K. P., Fansiria, T., Carrington, L. B., Kramer, L. D., Thomas, M. B. and Scott, T. W. (2011) Impact of daily temperature fluctuations on dengue virus transmission by *Aedes aegypti*. *Proc. Natl. Acad. Sci. USA* **108**, 7460-7465.

Landa, K. (1992). Seasonal declines in offspring fitness and selection for early reproduction in nymph-overwintering grasshoppers. *Evolution* **46**, 121-135.

Langkilde, T. and Shine, R. (2006). How much stress do researchers inflict on their study animals? A case study using a scincid lizard, *Eulamprus heatwolei*. *J. Exp. Biol.* **209**, 1035-1043.

Les, H. L., Paitz, R. T. and Bowden, R. M. (2009). Living at extremes: development at the edges of viable temperature under constant and fluctuating conditions. *Physiol. Biochem. Zool.* **82**, 105-112.

Luo, L. G., Ding, G. H. and Ji, X. (2010). Income breeding and temperature-induced plasticity in reproductive traits in lizards. *J. Exp. Biol.* **213**, 2073-2078.

Medonça, M. T. (1987). Photothermal effects on the ovarian cycle of the musk turtle, *Sternotherus odoratus*. *Herpetologica* **43**, 82-90.

Nelson, N. J., Thompson, M. B., Pledger, S., Keall, S. N. and Daughtery, C. H. (2004). Egg mass determines hatchling size, and incubation temperature influences post-hatching growth, of tuatara *Sphenodon punctatus*. *J. Zool.* **263**, 77-87.

Nussbaum R. A., Brodie, E. D., Jr and Storm, R. M. (1983). *Amphibians and Reptiles of the Pacific Northwest*. Moscow: University of Idaho Press.

Olsson, M., Wapstra, E., Schwartz, T., Madsen, T., Ujvari, B. and Uller, T. (2010). In hot pursuit: fluctuating mating system and sexual selection in sand lizards. *Evolution* **65**, 574-583.

Oufiero, C. E. and Angilletta, M. J., Jr (2010). Energetics of lizard embryos at fluctuating temperatures. *Physiol. Biochem. Zool.* **83**, 869-876.

Paaijmans, K. P., Blanford, S., Bell, A. S., Blanford, J. I., Read, A. F. and Thomas, M. B. (2010). Influence of climate on malaria transmission depends on daily temperature variation. *Proc. Natl. Acad. Sci. USA* **107**, 15135-15139.

Parmesan, C. (2006). Ecological and evolutionary responses to recent climate change. *Annu. Rev. Ecol. Evol. Syst.* **37**, 637-669.

Parmesan, C. (2007). Influence of species, latitudes and methodologies on estimate of phenological response to global warming. *Glob. Change Biol.* **13**, 1860-1872.

Pedhazur, E. J. (1982). *Multiple Regression in Behavioral Research*, 2nd Edn. Fort Worth: Harcourt Brace College Publishers.

Pounds, J. A. (2001). Climate and amphibian declines. *Nature* **410**, 639-640.

Qualls, C. P. and Andrews, R. M. (1999). Maternal body volume constrains water uptake by lizard eggs *in utero*. *Funct. Ecol.* **13**, 845-851.

Rillig, M. C., Wright, S. F., Shaw, M. R. and Field, C. B. (2002). Artificial climate warming positively affects arbuscular mycorrhizae but decreases soil aggregate water stability in an annual grassland. *Oikos* **97**, 52-58.

Rohr, J. R. and Raffel, T. R. (2010). Linking global climate and temperature variability to widespread amphibian declines putatively caused by disease. *Proc. Natl. Acad. Sci. USA* **107**, 8269-8274.

Root, T. L., Price, J. T., Hall, K. T., Schneider, S. H., Rosenzweig, C. and Pounds, J. A. (2003). Fingerprints of global warming on wild animals and plants. *Nature* **421**, 57-60.

Schultz, E. T. (1993). The effects of birth date on fitness of female dwarf perch, *Micrometrus minimus* (Perciformes: Embiotocidae). *Evolution* **47**, 520-539.

Schwanz, L. E. and Janzen, F. J. (2008). Climate change and temperature-dependent sex determination: can individual plasticity in nesting phenology prevent extreme sex ratios? *Physiol. Biochem. Zool.* **81**, 826-834.

Shine, R. (1980). 'Costs' of reproduction in reptiles. *Oecologia* **46**, 92-100.

Shine, R. (1995). A new hypothesis for the evolution of viviparity in reptiles. *Am. Nat.* **160**, 582-593.

Shine, R. (2002). Eggs in autumn: responses to declining incubation temperatures by the eggs of montane lizards. *Biol. J. Linn. Soc.* **76**, 71-77.

Shine, R. (2004). Seasonal shifts in nest temperature can modify the phenotypes of hatchling lizards, regardless of overall mean incubation temperature. *Funct. Ecol.* **18**, 43-49.

Shine, R. and Harlow, P. (1993). Maternal thermoregulation influences offspring viability in a viviparous lizard. *Oecologia* **96**, 122-127.

Shine, R., Elphick, M. J. and Harlow, P. S. (1997). The influence of natural incubation environments on the phenotypic traits of hatchling lizards. *Ecology* **78**, 2559-2568.

Sinervo, B. and Calsbeek, R. (2003). Physiological epistasis, ontogenetic conflict and natural selection on physiology and life history. *Integr. Comp. Biol.* **43**, 419-431.

Sinervo, B. and Doughty, P. (1996). Interactive effects of offspring size and timing of reproduction on offspring reproduction: experimental maternal, and quantitative genetic aspects. *Evolution* **50**, 1314-1327.

Sinervo, B., Svennson, E., and Comendant, T. (2000). Density cycles and an offspring quantity and quality game driven by natural selection. *Nature* **406**, 985-988.

Sinervo, B., Méndez-de-la-Cruz, F., Miles, D. B., Heulin, B., Bastiaans, E., Villagrán-Santa Cruz, M., Lara-Resendiz, R., Martínez-Méndez, N., Calderon-Espinosa, M. L., Meza-Lázaro, R. N., Jr et al. (2010). Erosion of lizard diversity by climate change and altered thermal niches. *Science* **328**, 894-899.

Telemeco, R. S., Radder, R. S., Baird, T. A. and Shine, R. (2010). Thermal effects on reptile reproduction: adaptation and phenotypic plasticity in a montane lizard. *Biol. J. Linn. Soc.* **100**, 642-655.

Tinkle, D. W. (1967). The life and demography of the side-blotched lizard, *Uta stansburiana. Misc. Publ. Mus. Zool. Univ. Michigan* **132**, 1-182.

Tinkle, D. W. and Irwin, L. N. (1965). Lizard reproduction – refractory period and response to warmth in *Uta stansburiana* females. *Science* **148**, 1613-1614.

Verhulst, S. (1998). Multiple breeding in the great tit, II. The costs of rearing a second clutch. *Funct. Ecol.* **12**, 132-140.

Vitt, L. J. and Congdon, J. D. (1978). Body shape, reproductive effort, and relative clutch mass in lizards: resolution of a paradox. *Am. Nat.* **112**, 595-608.

Wapstra, E. (2000). Maternal basking opportunity affects juvenile phenotype in a viviparous lizard. *Funct. Ecol.* **14**, 345-352.

Warner, D. A. and Shine, R. (2007). Fitness of juvenile lizards depends on seasonal timing of hatching, not offspring body size. *Oecologia* **154**, 65-73.

Warner, D. A., Woo, L. K., Van Dyk, D. A., Evans, C. S. and Shine, R. (2010). Egg incubation temperature affects male reproductive success but not display behaviors in lizards. *Behav. Ecol. Sociobiol.* **64**, 803-813.

Weisrock, D. W. and Janzen, F. J. (1999). Thermal and fitness-related consequences of nest location in painted turtles (*Chrysemys picta*). *Funct. Ecol.* **13**, 94-101.

Whitney-Johnson, A., Thompson, M. and Hon, E. (2005). Responses to predicted global warming in *Pieris rapae* L. (Lepidoptera): consequences of nocturnal versus diurnal temperature change on fitness components. *Environ. Entomol.* **34**, 535-540.

Zani, P. A. (2005). Life-history strategies near the limits of persistence: winter survivorship and spring reproduction in the common side-blotched lizard (*Uta stansburiana*) in eastern Oregon. *J. Herpetol.* **39**, 166-169.

Zani, P. A. (2008). Climate change trade-offs in the side-blotched lizard (*Uta stansburiana*): effects of growing-season length and mild temperatures on winter survival. *Physiol. Biochem. Zool.* **81**, 797-809.

Zani, P. A. and Rollyson, M. E. (2011). The effects of climate modes on growing-season length and timing of reproduction in the Pacific Northwest as revealed by biophysical modeling of lizards. *Am. Midl. Nat.* **165**, 372-388.

Zani, P. A., Swanson, S. E. T., Corbin, D., Cohnstaedt, L. W., Agotsch, M. D., Bradshaw, W. E. and Holzapfel, C. M. (2005). Geographic variation in thermal tolerance of transient thermal stress in the mosquito *Wyeomyia smithii. Ecology* **86**, 1206-1211.

Zani, P. A., Neuhaus, R. A., Jones, T. D. and Milgrom, J. E. (2008). Effects of reproductive burden on endurance performance in side-blotched lizards (*Uta stansburiana*). *J. Herpetol.* **42**, 76-81.