

## ORIGINAL ARTICLE

# Extreme developmental temperatures result in morphological abnormalities in painted turtles (*Chrysemys picta*): a climate change perspective

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## Abstract

Increases in extreme environmental events are predicted to be major results of ongoing global climate change and may impact the persistence of species. We examined the effects of heat and cold waves during embryonic development of painted turtles (*Chrysemys picta*) in natural nests on the occurrence of abnormal shell morphologies in hatchlings. We found that nests exposed to extreme hot temperatures for >60 h produced more hatchlings with abnormalities than nests exposed to extreme hot temperatures for shorter periods, regardless of whether or not nesting females displayed abnormal morphologies. We observed no effect of extreme cold nest temperatures on the occurrence of hatchlings with abnormalities. Moreover, the frequency of nesting females with abnormal shell morphologies was approximately 2-fold lower than that of their offspring, suggesting that such abnormalities are negatively correlated with survival and fitness. Female turtles could potentially buffer their offspring from extreme heat by altering aspects of nesting behavior, such as choosing shadier nesting sites. We addressed this hypothesis by examining the effects of shade cover on extreme nest temperatures and the occurrence of hatchling abnormalities. While shade cover was negatively correlated with the occurrence of extreme hot nest temperatures, it was not significantly correlated with abnormalities. Therefore, female choice of shade cover does not appear to be a viable target for selection to reduce hatchling abnormalities. Our results suggest that increases in the frequency and intensity of heat waves associated with climate change might perturb developmental programs and thereby reduce the fitness of entire cohorts of turtles.

**Key words:** anomaly, fundamental niche, incubation, nesting behavior, scute deformity

## INTRODUCTION

Organisms are intimately connected to their environments, with factors such as temperature and precipita-

tion defining major axes of the fundamental niche for many, if not most, species (Hutchinson 1957; Futuyma 2001; Angilletta 2009). Because environments vary spatially and temporally, coping with environmental variation is a major challenge for all organisms (Townsend *et al.* 2003; Piersma & van Gils 2011). An increasingly important source of such environmental variation is global climate change (e.g. Parmesan 2006; Visser 2008; Telemeco *et al.* 2009). Long term changes in both temperature and hydrology have already occurred at a range

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of geographic scales (Williams *et al.* 2003; IPCC 2007; Pryor & Schoof 2008), and these changes will continue to a degree dependent upon future global socioeconomic policy (IPCC 2007; Moss *et al.* 2008; Bloom 2010). Conservative estimates predict increases in global temperature of 2.0–8.0 °C, **along with substantial changes** in precipitation, over the next century, with some regions affected more than others (IPCC 2007; Moss *et al.* 2008; Moser *et al.* 2009; Angilletta & Sears 2011). Importantly, climate change is predicted to increase thermal and hydric variance in addition to directional changes in the means of these factors (IPCC 2007; Moser *et al.* 2009; Bloom 2010; Takle 2011). An important consequence of increasing climatic variance is higher frequencies and intensities of extreme environmental events (EEEs), such as droughts, floods and heat waves (Saunders *et al.* 2008; Bloom 2010; Takle 2011).

Over the past 2 decades, much work has described the effects of climate change on biota (reviewed in Parmesan 2006; Visser 2008; Angilletta 2009; Bloom 2010; Sears & Angilletta 2011). Changes in phenology (timing of life-history events), morphology, physiology, distribution and abundance have all been reported in a wide range of taxa in response to recent climate change (e.g. Janzen 1994a; Visser & Both 2005; Araújo *et al.* 2006; Parmesan 2006; Telemeco *et al.* 2009; Sears & Angilletta 2011). These studies have generally assessed how changes in mean environmental conditions affect organisms, with few field studies examining the effects of climatic variance (some exceptions include Shine *et al.* 2003 and Rochester *et al.* 2010). However, increases in climatic variance might have large impacts on biota beyond those of changing means, because EEEs could temporarily shift an organism outside of its fundamental niche (Jackson *et al.* 2009; Angilletta & Sears 2011; Clusella-Trullas *et al.* 2011). Altering the niche will be deleterious if individuals are unable to buffer themselves from these changes. Given the stochastic nature and speed of onset of many EEEs, only the most highly mobile species should escape their effects. Therefore, the increased frequency and intensity of EEEs expected under climate change might have profound negative effects on many populations, communities and ecosystems (Jackson *et al.* 2009; Angilletta & Sears 2011).

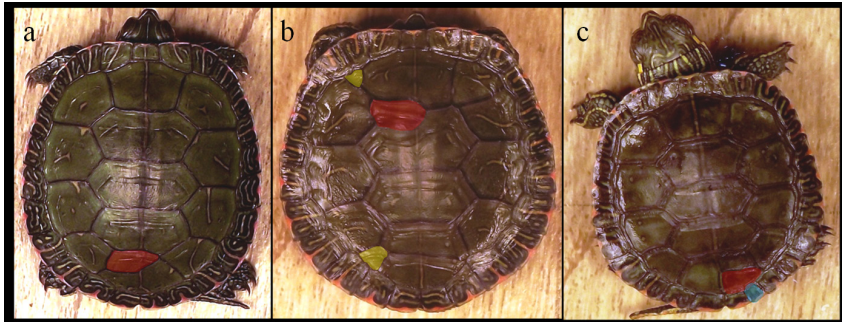
Species that are least able to control their hydric or thermal physiology may be at greatest risk of decline resulting from climate change (Janzen 1994a; Araújo *et al.* 2006; Bloom 2010; Milanovich *et al.* 2010). For example, reptiles, like all ectotherms, rely on temperature for all metabolic activity (Angilletta 2009; Vitt & Caldwell

2009), and temperatures to which reptile embryos are exposed during development profoundly affect offspring phenotype and fitness (i.e. survivorship, behavior, morphology, physiology and sex; Bull 1980; Shine 2005; Booth 2006; Warner & Shine 2008; Schwanz *et al.* 2010; Telemeco *et al.* 2010). Moreover, in oviparous species, developing embryos cannot behaviorally buffer themselves as free-ranging individuals do. As a result of these sensitivities, many reptile species have already shown signs of climate-change induced decline (e.g. Araújo *et al.* 2006; Reading *et al.* 2010; Sinervo *et al.* 2010). To abate the negative effects of climate change on such species, we must understand the factors most responsible for these declines and how such changes affect the biology of organisms.

To advance this understanding, we assessed the effects of thermal variation on painted turtles (*Chrysemys picta* Schneider, 1783). Specifically, we examined how extreme temperatures, like those that might result from heat or cold waves, in natural nests relate to the incidence of morphological abnormalities in hatchlings. A small percentage of individuals in most (if not all) species develop abnormally (e.g. Zangerl 1969; Gilbert & Epel 2009; Löwenborg *et al.* 2011). These abnormalities result from genetic or environmental effects, and generally reduce fitness (Gilbert & Epel 2009). In the laboratory, exposure to extreme incubation temperatures increases the frequency of abnormalities in numerous reptile species, suggesting that these abnormalities result from exposure to suboptimal developmental temperatures (Yntema 1960; Braña & Ji 2000; Löwenborg *et al.* 2011). If so, increased occurrence of EEEs might elevate frequencies of developmental abnormalities and, thus, reduce fitness in many reptile species. However, conclusions about the effects of EEEs are difficult to draw from laboratory studies alone because extreme conditions generally differ in duration and intensity compared to those encountered in natural nests. Therefore, to test the hypothesis that extreme developmental temperatures induce morphological abnormalities in nature, we explored relationships between extreme temperatures in *C. picta* nests and morphological abnormalities (carapace scute deformities, Fig. 1) in hatchlings. In addition, we compared the incidence of morphological abnormalities in nesting females and their offspring to test the hypotheses that: (i) scute deformities are heritable and (ii) scute deformities are negatively correlated with survival.

Phenotypes that minimize exposure of developing embryos to extreme temperatures, such as variations in nesting behavior, should be prime targets for natural se-

**Figure 1** Common deformities of carapace scutes in hatchling painted turtles (*Chrysemys picta*). Additional/misformed vertebral scutes, costal scutes, and marginal scutes are outlined in red, yellow and blue, respectively: (a) has an extra vertebral scute; (b) has an extra vertebral scute and 2 extra/misformed costal scutes; and (c) has an extra vertebral scute and right marginal scute. Normal individuals have 5 vertebral scutes, 4 costal scutes, 1 nuchal scute and 24 marginal scutes (12 on each side).



lection if exposure to extreme temperatures has fitness consequences (Visser 2008; Telemeco *et al.* 2009; Refsnider & Janzen 2010). For example, female turtles could reduce the probability that their eggs are exposed to extreme temperatures by choosing more heavily shaded nest sites (Janzen & Morjan 2001; Doody *et al.* 2006; Telemeco *et al.* 2009). Therefore, we also examined the relationship between the amount of vegetation cover over nests at oviposition and extreme nest temperatures during development to test the plausibility of females buffering their offspring from extreme temperatures by altering nest-site choice. The results from this study provide information on the sensitivity of turtle embryos to extreme perturbations in the natural thermal environment, and, therefore, on how sensitive populations might be to increased variance in thermal conditions resulting from climate change.

## MATERIALS AND METHODS

### Data collection

We examined the effects of extreme temperatures on the incidence of morphological abnormalities as part of our long term study of painted turtles in the Thomson Causeway Recreation Area (TCRA) in Carroll County, Illinois (41°57'N, 90°07'W). As a result of variations in our standard protocol over time and numerous years with extreme nest predation, the data necessary for the present study were only available for years 2001–2003. Our general field methods are described in detail elsewhere (e.g. Weisrock & Janzen 1999; Morjan 2003a; Schwanz *et al.* 2010; Warner *et al.* 2010). Briefly, for the present study, we monitored female turtles during the nesting season (May and Jun) as they emerged from

aquatic habitat to nest on land (2001  $N = 241$ , 2002  $N = 387$ , 2003  $N = 327$ ; Table 1). After females completed oviposition, we examined their carapaces for abnormalities (i.e. missing or additional scutes). In addition, we removed all eggs from nests, then counted and carefully returned them to the nests in their original order/configuration. Handling painted turtle eggs immediately following oviposition has no effect on survivorship in both the field (Samson *et al.* 2007) and the laboratory (Drajeske 1974; Ewert 1979; Feldman 1983). Even so, we gently handled eggs and took care to ensure that eggs were not inverted when returned to their nest cavities. At this time, we also placed data loggers (iButton, Dallas Semiconductor, Dallas, TX, USA) in the center of a random subset of nests (2001  $N = 16$ , 2002  $N = 35$ , 2003  $N = 72$ ; Table 1). The loggers recorded hourly temperatures for 70 days following oviposition, which encompasses approximately 85% of embryonic development in TCRA painted turtles (mean incubation length = 81.2 days, range = 65–98 days,  $N = 233$  hatchlings) (Mitchell *et al.* 2013). For each nest, we also measured vegetation cover in each of the 4 cardinal directions using a spherical densiometer (see Janzen 1994b). We used the sum of south and west percentage vegetation cover for analyses because this best predicts individual nest conditions in the TCRA (Janzen 1994b).

In Sep, we excavated the nests once again, and retrieved hatchlings and loggers for transport to Iowa State University. We were able to sample all offspring in this manner because neonatal *C. picta* remain in the nest in the TCRA until spring. In the laboratory, we downloaded temperature data from the loggers, and examined hatchling carapaces for scute abnormalities (Fig. 1) as we had with the adult females. We chose to assess scute abnormalities because they are readily observed (Lynn

**Table 1** Painted turtle (*Chrysemys picta*) nests at the Thomson Causeway Recreation Area (TCRA) in Illinois, USA. Number of nests constructed during the 2001, 2002 and 2003 nesting seasons and number of nests for which complete data were available for the present study are shown. For the latter, information on maternal abnormalities, number of eggs laid, hatchling survivorship and the number of hatchlings born with abnormalities are given.

	2001	2002	2003	Total
All nests				
Nests constructed	241	387	327	955
Nests with temperature data (full)	16	35	72	123
Nests with temperature data (not depredated)	8	33	72	113
113 nests with complete data				
Nests constructed by abnormal females	3	4	9	16
Eggs laid	125	359	728	1212
Surviving hatchlings	86	222	236	544
Abnormal hatchlings	10	78	64	152

& Ullrich 1950; Zangerl 1969; Ewert 1979) and likely correlate negatively with fitness (Ewert 1979; Mast & Carr 1989; F. J. Janzen, pers. observ.). For analyses, we quantified the frequency of nesting females with shell abnormalities, the frequency of their hatchlings with abnormalities, and the frequency of eggs per nest that produced hatchlings with shell abnormalities.

After quantifying shell abnormalities, we identified the sex of hatchlings from a subset of nests (2001  $N = 25$ , 2002  $N = 206$ , 2003  $N = 173$ ; includes all nests with loggers and others) by examining gonads. Because painted turtles have temperature-dependent sex determination (TSD) (Janzen & Paukstis 1991), the sex ratio for each annual hatchling cohort should indicate the relative thermal conditions to which the cohort was exposed. In addition, the pivotal temperature (temperature that produces a 1:1 sex ratio) maximizes hatching success in painted turtles. Therefore, to conservatively estimate extreme hot and extreme cold nest temperatures for painted turtle development, we examined temperature data from years that produced an approximate 1:1 sex ratio. Including male-biased (colder than optimum) or female-biased (warmer than optimum) years, unless perfectly balanced, would bias these estimates. Moreover, sex ratio theory predicts that years with 1:1 hatchling sex ratios should represent evolutionarily average thermal conditions (e.g. Fisher 1930; Bull & Charnov 1988; Janzen & Phillips 2006).

Estimating extreme temperature thresholds for painted turtle development from nest data is likely more biologically relevant than examining recent averages in air

temperature because the latter may differ substantially from conditions in nests (Ackerman & Lott 2004) and might be influenced by sampling biases (e.g. all data collected after the onset of climate change, often only collected at a few points throughout the day). While examining long term averages from natural nests could remove bias and provide a clearer picture of the thermal conditions to which painted turtle embryos are exposed, we do not have complete thermal profiles for nests over long periods. Therefore, examining nest temperatures within the years of our study that produced a 1:1 sex ratio should provide the best available estimate of what constitutes 'extreme conditions' in natural painted turtle nests in the TCRA.

### Data analysis

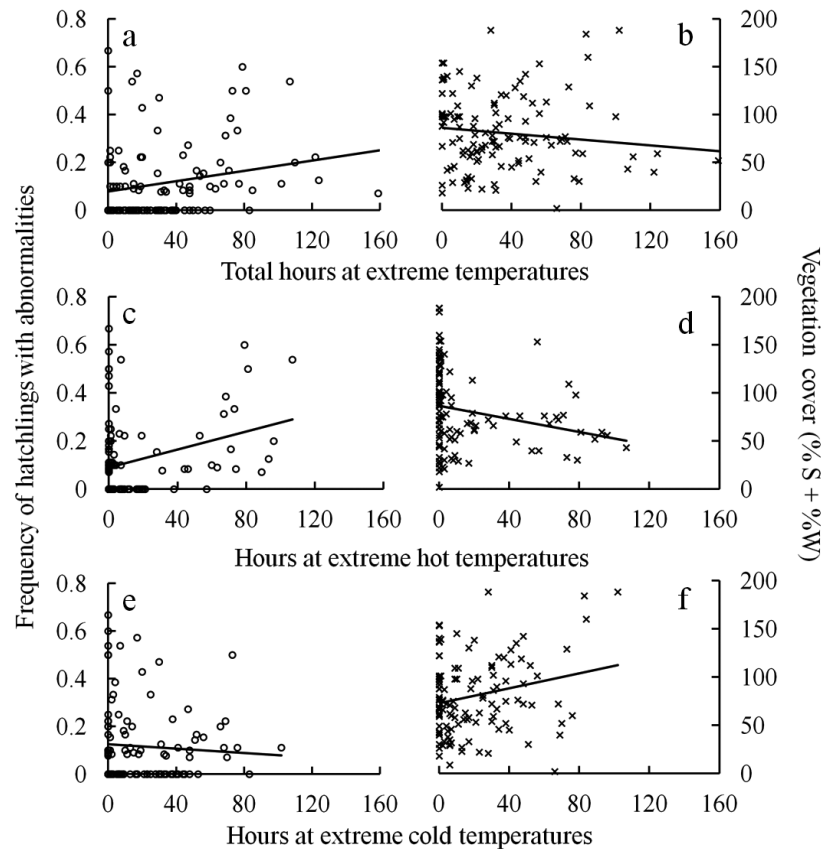
Data were available on maternal and hatchling abnormalities, nest temperature and vegetation cover for 113 nests (2001  $N = 8$ , 2002  $N = 33$ , 2003  $N = 72$ ; Table 1, sample sizes in 2001 and 2002 are lower than the number of nests with thermal data loggers in these years because some nests were depredated); data for each nest were our unit of replication for analyses. Because offspring sex ratios were approximately 1:1 in 2001 and 2002, we used nest temperature data from these years to conservatively calculate extreme temperature thresholds. We first calculated a grand mean temperature from the pooled 2001 and 2002 nest temperature data, denoting extreme temperatures as those  $>2$  SD below or above the grand mean. We then quantified the number of hours each nest spent at extreme temperatures.

To explore whether hatchling abnormalities were more intrinsically or environmentally induced, we used a  $\chi^2$  analysis to examine whether abnormal females were more likely to produce nests with at least 1 abnormal hatchling than were normal females. We then used linear regression analyses to examine the effects of time (number of hours) that nests spent at extreme temperatures (cold, hot and total cold + hot) on the frequency, per clutch, of hatchlings with abnormalities. To better meet the assumptions of parametric statistics, we arcsine-transformed the frequency of abnormalities. In addition, 1 nest was an outlier (exposed to 2.2 times more extreme hot hours than any other nest and 75% of eggs produced offspring with abnormalities) and, thus, we removed it from analyses. Inclusion of this nest, however, did not qualitatively affect conclusions. We used linear regression analyses to assess the effect of vegetation cover over nests at oviposition on the number of hours that nests spent at extreme temperatures (cold, hot and

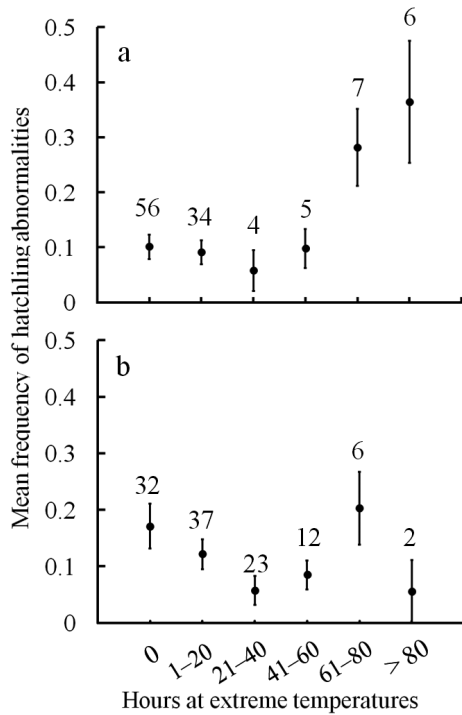
total cold + hot). Finally, we examined the correlation between vegetation cover over nests and the frequency of abnormal hatchlings produced by those nests.

## RESULTS

Sex ratios in 2001, 2002 and 2003 (measured as percent male) were 50.6, 53.2 and 79.5%, respectively (mean sex ratio = 61.1%, the long term sex ratio in the TCRA is 61.8%) (Schwanz *et al.* 2010). Because hatchling sex ratios in 2001 and 2002 were approximately 1:1, we considered nest temperature during these 2 years to represent relatively optimum conditions for development. By contrast, the highly male-biased sex ratio in 2003 indicates cooler than optimum nest temperatures. The 2001 and 2002 grand mean nest temperature and SD were 26.0 and 4.1 °C, respectively, whereas the 2003 mean nest temperature and SD were 23.8 and 3.5 °C, respectively. Extreme temperatures (defined as being 2 SD or more from the 2001 + 2002 grand mean)



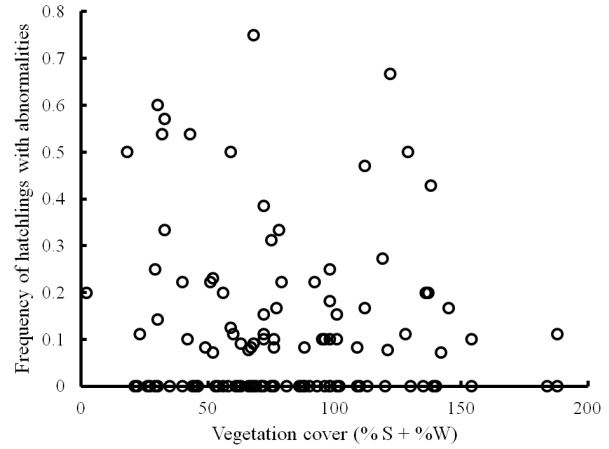
**Figure 2** Correlations between the number of hours that painted turtle (*Chrysemys picta*) nests spent at extreme temperatures during embryonic development and the frequency of hatchling abnormalities in nests (a,c,e), and vegetation cover over nests at oviposition (sum of south and west percent vegetation cover: b,d,f). Extreme temperatures are all those greater than 2 SD from the mean (>34.2 and <17.8 °C, respectively). Each point represents 1 nest, and lines are minimum sum of squares regressions.



**Figure 3** Effect of the number of hours that painted turtle (*Chrysemys picta*) nests spent at extreme hot (a) and extreme cold (b) temperatures during embryonic development on the frequency of hatchlings with abnormalities. Extreme temperatures are all those greater than 2 SD from the mean (>34.2 and <17.8 °C, respectively). Mean frequencies  $\pm$  SE in 20 h bins are shown. Numbers above error bars are sample sizes per bin.

were nest temperatures <17.8 °C (extreme cold) and >34.2 °C (extreme hot).

In average years (2001 and 2002), 17.0% of nesting females displayed abnormal shell morphologies whereas 28.6% of hatchlings were abnormal (Table 1). Seventy-eight percent of nests examined during these years produced at least 1 abnormal hatchling. In the cooler year (2003), 12.5% of females displayed abnormal shell morphologies whereas 27.1% of hatchlings were abnormal (Table 1). In this year, 37.5% of nests examined produced at least 1 abnormal hatchling. In total, 14.2% of nesting females and 27.9% of hatchlings displayed abnormal shell morphologies (Table 1). Abnormal females were not more likely to produce a nest with at least 1 abnormal hatchling than were normal females ( $\chi^2 = 1.260$ ,  $P = 0.26$ ).



**Figure 4** Effect of vegetation cover over painted turtle (*Chrysemys picta*) nests at oviposition on the frequency of hatchlings with abnormalities. Each point represents 1 nest. Vegetation cover is the sum of south and west percent vegetation cover.

Linear regression analyses revealed a positive correlation ( $F_{1,109} = 8.42$ ,  $P = 0.0045$ ,  $R^2 = 0.06$ ) between the total number of hours that eggs were exposed to extreme temperatures and the frequency of hatchling abnormalities (Fig. 2a). However, when the number of hours exposed to hot and cold extreme temperatures were analyzed separately, only extreme hot hours was correlated with abnormality frequency, and this correlation was positive (extreme hot:  $F_{1,109} = 13.66$ ,  $P = 0.0003$ ,  $R^2 = 0.10$ ; extreme cold:  $F_{1,109} = 0.02$ ,  $P = 0.89$ ,  $R^2 = 0.0002$ ; Fig. 2c,e). In addition, the relationship between exposure to extreme temperatures and hatchling abnormalities appeared to be nonlinear. When the frequency of abnormalities was binned by hours at extreme temperatures (Fig. 3), the frequency of hatchling abnormalities did not increase significantly until embryos were exposed to extreme hot temperatures for at least 60 h. Assuming an 81 day incubation period, this constitutes merely 3.1% of embryonic development.

Linear regression also revealed correlations between vegetation cover and the number of hours that nests reach extreme temperatures (total extreme:  $F_{1,120} = 3.01$ ,  $P = 0.09$ ,  $R^2 = 0.02$ ; extreme hot:  $F_{1,120} = 11.60$ ,  $P = 0.0009$ ,  $R^2 = 0.09$ ; extreme cold:  $F_{1,120} = 6.26$ ,  $P = 0.0137$ ,  $R^2 = 0.05$ ; Fig. 2b,d,f). Accordingly, vegetation cover was negatively correlated with extreme

hot temperatures and positively correlated with extreme cold temperatures (Fig. 2d,f). Even so, vegetation cover did not predict the frequency of hatchling abnormalities in nests ( $F_{1,109} = 0.37$ ,  $P = 0.55$ ,  $R^2 = 0.003$ ; Fig. 4).

## DISCUSSION

### Relationship between scute deformities and fitness

Because environmental factors directly affect development in ectotherms (Birchard 2004; Deeming 2004; Angilletta 2009; Gilbert & Epel 2009), exposure of embryos to suboptimal incubation conditions can perturb development, producing abnormal phenotypes and/or death (Lynn & Ullrich 1950; Arnold & Peterson 2002; Birchard 2004; Gilbert & Epel 2009). A commonly studied example of sub-lethal abnormalities that can be influenced by the developmental environment is asymmetry (Møller 1997; Clarke 1998; Lens *et al.* 2002; Shine *et al.* 2005). Although the impacts of asymmetry are difficult to generalize (Clarke 1998; Lens *et al.* 2002), asymmetry negatively correlates with fitness in many cases (Møller 1997; Lens *et al.* 2002; Löwenborg *et al.* 2011). Scute deformities, which frequently manifest as asymmetry (i.e. greater number of marginal or costal scutes on 1 side), are the most commonly observed phenotypic abnormality in turtles (Zangerl 1969; Ewert 1979; MacCulloch 1981; Mast & Carr 1989).

Even though previous authors have asserted that scute deformities negatively correlate with fitness (e.g. Ewert 1979), we are aware of only 1 previous study that examined this correlation. In Kemp's ridley sea turtles (*Lepidochelys kempii* Garman, 1880), scute deformities are more common in embryos that fail to hatch than in those that survive (Mast & Carr 1989). Our result that, proportionally, approximately half as many nesting females displayed scute deformities as their offspring provides additional support for the hypothesis that individuals with scute deformities are less likely to survive to maturity than are normal individuals. However, we were unable to examine males in the parental generation. Still, this sampling bias likely resulted in our estimated difference between the frequency of scute deformities in the adult and juvenile generations being conservative because warm temperatures induce development of both females and deformed scutes. A greater issue is the assumption that both generations had similar frequencies of scute deformities at hatching. We are currently unable to test the validity of this assumption. Further re-

search testing the hypothesis that scute deformities correlate with fitness in turtles is clearly needed.

Due to the integrative nature of vertebrate development and the general sensitivity of developmental processes to environmental conditions, external stimuli that alter 1 aspect of development likely alter others (Gilbert & Epel 2009). Thus, deformed scutes in turtles, while superficial, likely indicate additional perturbations to development that directly affect survival and reproduction. For example, a superficial abnormality induced by extreme environmental temperatures in snakes (additional ventral scales) indicates an underlying osteological deformity (extra rib) and is negatively correlated with performance and fitness (Arnold & Peterson 2002; Shine *et al.* 2005; Löwenborg *et al.* 2011). The integrated nature of vertebrate development (Gilbert & Epel 2009), the frequent negative correlation between abnormal phenotypes and fitness in other taxa (Møller 1997; Lens *et al.* 2002; Löwenborg *et al.* 2011) and the reduced frequency of scute abnormalities in females compared with their offspring that we observed jointly suggest that scute abnormalities in turtles likely correlate negatively with fitness.

### Effects of extreme environmental temperatures on scute deformities

Previous laboratory analyses suggest that extreme developmental environments can induce scute deformities (Lynn & Ullrich 1950; Yntema 1960). Our observations corroborate these results in a field setting. The frequency of hatchling painted turtles with deformed scutes correlated positively with the length of time that nests spent at temperatures above 34.2 °C (extreme hot, >2 SD above the mean). This relationship appears to be a threshold-like response: extreme temperatures only induced substantial abnormal scute development after approximately 60 h of exposure. Interestingly, 34 °C is approximately the maximum temperature for embryonic development in painted turtles (Neuwald & Valenzuela 2011; Telemeco *et al.* 2013), with developmental rate rapidly decreasing to zero at higher temperatures. Extreme high temperatures likely reduce the rate of development by disrupting molecular interactions (e.g. enzyme-substrate and membrane dynamics) (Angilletta 2009; Gilbert & Epel 2009) and these same disruptions might be responsible for inducing abnormal phenotypes. Because 34 °C marks both the maximum temperature for active embryonic development and an apparent threshold for inducing abnormalities, we suggest that this temperature is approximately the maximum bound-

ary of the thermal fundamental niche in painted turtle embryos.

Why painted turtle embryos required >60 h of exposure to extreme heat before scute morphology was affected is not fully understood, but we propose several plausible explanations for the observed pattern. First, development might be buffered against minor perturbations, with a minimum dosage of extreme temperatures being required to significantly affect development. Alternatively, length of exposure might be correlated with an additional factor that is directly responsible for the observed pattern. For example, nests exposed longer to extreme hot temperatures likely also experience hotter maximum temperatures than nests that are exposed for fewer hours. In addition, if a specific period of development is sensitive to high temperatures, then nests exposed longer to extremes are more likely to be exposed during the sensitive period. Further data are needed to directly test these alternative hypotheses.

By contrast, we observed no impact of extreme cold temperatures on the frequency of scute abnormalities in hatchling painted turtles. This result differs from work on the common snapping turtle (*Chelydra serpentina* Linnaeus, 1758), which found a strong effect of extreme cold incubation temperature in the laboratory on the occurrence of shell abnormalities (Yntema 1960). This discrepancy might result from differences in developmental sensitivities among these species and/or from differences in exposure. To induce abnormal development, Yntema (1960) exposed embryos to 15.0 °C for 3 weeks early in development. The natural nests that we examined rarely reached such low temperatures: the most extreme exposure that we observed was a nest that spent approximately 100 h (approximately 4 days) below 17.8 °C (2 SD below the grand mean), which is roughly 5 times shorter than the duration needed to observe abnormal development in snapping turtles. However, in Saskatchewan, near the northern extreme of the painted turtle range, adults display abnormally high frequencies of scute deformities (MacCulloch 1981), suggesting that extreme cold temperatures might impact painted turtle development in some parts of their range. Thus, if the painted turtle nests that we monitored had maintained extreme cold temperatures for longer periods, we too might have observed abnormal development, but such temperature regimes are probably not biologically relevant in the TCRA.

Even though exposure to extreme warm nest temperatures correlates significantly with developmental abnormalities in hatchling painted turtles, it is important to note that this relationship only explained 10% of

the variation in abnormalities that we observed. Many nests had low frequencies of abnormalities even when exposed to extreme hot temperatures for long periods, whereas others showed high frequencies of abnormalities even though they were never exposed to extreme temperatures. Much of this variation may result from the crudeness with which we measured exposure to extreme temperatures (hours spent >2 SD above or below the mean). Importantly, the remaining variation likely results from environmental factors rather than heritable variation because females with deformed scutes were not more likely to produce deformed hatchlings than were normal females. Hydric conditions are one such environmental factor, and profoundly affect embryonic development in reptiles (Packard 1999; Flatt *et al.* 2001; Warner *et al.* 2012). However, a major effect of soil water potential seems unlikely because soil hydric conditions in this region are relatively stable (Ackerman & Lott 2004) and other aspects of hatchling morphology are unaffected by natural variation in hydric conditions in the TCRA (Warner *et al.* 2010). Additional factors that might be responsible for this variation in the frequency of developmental abnormalities include variation in *de novo* mutation, soil type, physical attributes of the nest and individual thermal sensitivity.

### Climate change implications

Increases in the frequency and intensity of EEEs are major effects predicted of ongoing global climate change (IPCC 2007). Heat waves, droughts and floods have increased around the globe and these increases are predicted to continue (IPCC 2007; Saunders *et al.* 2008; Moser *et al.* 2009; Takle 2011). Recently, the effects of increased EEEs on human health, welfare and infrastructure have begun to be considered (Moser *et al.* 2009; Takle 2011). However, our understanding of the ecological effects of increases in EEEs associated with climate change is poor (Jackson *et al.* 2009; Angilletta & Sears 2011; Clusella-Trullas *et al.* 2011). Because EEEs have the potential to rapidly shift individuals outside of their fundamental niches (Angilletta 2009; Jackson *et al.* 2009), changes in EEEs might be more important for species' persistence than changes in environmental means. We show that heat waves could disrupt painted turtle development and thereby potentially reduce the fitness of entire cohorts of hatchlings. Eggs need only be exposed to temperatures over 34.2 °C for 60 h (2.5 days) for marked increases in abnormal development to become apparent. Throughout much of the painted turtle range (central and eastern North America) (Conant & Collins 1998), such thermal regimes already occur with relative frequency (e.g. Durre & Wal-



lace 2000; Takle 2011). If the incidence of heat waves increases as a result of climate change, the frequency of hatchling painted turtles with abnormalities in the TCRA should also increase, even if mean temperatures remain relatively constant.

If exposure to extreme hot temperatures during development reduces offspring fitness (Angilletta 2009; Gilbert & Epel 2009), selection should act on female turtles to avoid nest sites that are likely to be exposed to such extremes. A mechanism on which selection might act is phenotypic plasticity in nesting behavior, observed in many reptile species (e.g. Morjan 2003b; Doody *et al.* 2006; Angilletta 2009; Telemeco *et al.* 2009; McGaugh *et al.* 2010), such that turtles nest in more thermally buffered (e.g. shadier) areas in years with elevated extreme hot temperatures. Such a mechanism would allow turtles to accommodate environmental variation, buffering populations from impending climate change. We tested the plausibility of this mechanism, finding that vegetation cover over nests was negatively correlated with the number of hours that nests experienced extreme hot temperatures, as expected. However, this correlation explained only 9.0% of the variance, and vegetation cover was not correlated with the frequency of hatchling abnormalities in nests. The lack of a correlation between vegetation cover and abnormalities suggests that selection might not act effectively on female choice of shade cover over nests to reduce hatchling abnormalities as climates change.

Alternatively, developmental sensitivity to extreme temperatures might directly evolve as global climate changes (Angilletta 2009; Clusella-Trullas *et al.* 2011; Schulte *et al.* 2011). As described above, 1 possible explanation for the low variation in hatchling abnormalities attributable to exposure to extreme heat is that developing embryos differ in their thermal sensitivities. Such variation in sensitivity to extreme temperatures might depend on genetic background and, thus, be heritable. If additive genetic variation in developmental sensitivity is present in the population, increases in heat wave frequency associated with climate change should strongly select for, and elicit microevolution of, individuals that are tolerant of high temperatures during development (Falconer & Mackay 1996; Clusella-Trullas *et al.* 2011). Such adaptive changes might enhance population persistence in the face of climate change.

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