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# Survival and reproductive costs of repeated acute glucocorticoid elevations in a captive, wild animal

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ABSTRACT

Organisms are continuously encountering both predictable and unpredictable ecological stressors within their environment. The activation of the hypothalamic-pituitaryadrenal (stress) axis is a fundamental process allowing animals to cope with and respond to such encounters. A main consequence of HPA axis activation is the release of glucocorticoid hormones. Although short-term glucocorticoid elevations lead to changes in physiological and behavioral processes that are often adaptive, our understanding of fitness consequences of repeated acute elevations in glucocorticoid hormones over a longer time period is largely lacking. This is of particular current importance as animals are facing a significant increase in exposure to stressors including those associated with human-induced rapid environmental change. Here, we test fitness-relevant consequences of repeated exposure to glucocorticoids in the absence of natural challenges, by treating wild-caught gravid female eastern fence lizards (*Sceloporus undulatus*) with a daily transdermal dose of a glucocorticoid hormone until laying. This treatment causes an increase in plasma glucocorticoids that mimics the natural response lizards have when they encounter a stressor in the wild, without confounding effects associated with the encounter itself. This treatment reduced females' reproductive success (hatching success) and survival. Further, glucocorticoid-induced reductions in reproductive success were greater when females had experienced higher temperatures the previous winter. This demonstrates the potential significant consequences of repeated exposure to acute elevations in glucocorticoid hormones. Additionally, the costs of repeated glucocorticoid elevation may be further exaggerated by an individual's previous experience, such as the potential compounding effects of winter warming increasing animals' vulnerability to increased glucocorticoid levels during spring breeding.

## 1. Introduction

Understanding how organisms respond to stressors in their environment is of increasing importance in a rapidly-changing world (Bijlsma and Loeschcke, 2005; Sih et al., 2011). An organism's response to environmental perturbations or challenges is mediated by the physiological "stress" response, a suite of neuroendocrine processes characterized by activation of the hypothalamic-pituitaryadrenal axis (HPA) and subsequent release of glucocorticoid hormones (Sapolsky, 2002). Glucocorticoid hormones function to simultaneously maintain physiological homeostasis and facilitate appropriate reactions to and recovery from environmental stressors (Wingfield et al., 1998; Sapolsky 2002; McEwen and Wingfield, 2003).

Although some definitions of stress and stressors necessitate a reduction in performance or fitness (Schulte, 2014), the physiological response to an ecological stressor – including glucocorticoid elevation – has generally been assumed to be an adaptive mechanism by which immediate survival is prioritized (Boonstra, 2013; Breuner et al., 2008; Sapolsky et al., 2000; Wingfield et al., 1998). Though some studies have reported negative fitness outcomes of chronic stressor exposure (Boonstra et al., 1998; Boonstra and Singleton, 1993; Bradley et al., 1980; Cyr and Michael Romero, 2007), there is limited evidence for fitness effects of repeated short-term elevations in glucocorticoids (Breuner et al., 2008, but see Cote et al., 2006), or direct relationships between ecologically meaningful concentrations of glucocorticoids and variation in fitness more generally (Bonier et al., 2017; Crespi et al., 2013; Hau et al., 2016).

Short-term elevations of glucocorticoid hormones have the potential

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to have lasting impacts on individuals. Frequent short-term increases in glucocorticoid hormones are likely to be energetically expensive, particularly given these hormones are directly associated with energy balance (Haase et al., 2016; Sapolsky et al., 2000). Glucocorticoids at high levels have been linked with reduced body weight and condition (De Vos et al., 1995; Klein, 2015); changes in food intake (Cote et al., 2006; Osborne, 2015); elevated rates of metabolism (DuRant et al., 2008; Haase et al., 2016); and suppressed immune function (McCormick et al., 2014). Further, studies have correlatively linked baseline glucocorticoid levels (Romero and Wikelski, 2010; Cabezas et al., 2007) and glucocorticoid reactivity (Blas et al., 2007) to variation in survival, while others have linked stressor-induced (predation risk) changes in glucocorticoids to changes in survival and reproduction (MacLeod et al., 2018; Zanette et al., 2011). Studies that have investigated direct links between glucocorticoids and fitness (in terms of survival and reproductive success) have largely used implants, which constantly release hormones and may not appropriately mimic the natural, short-term responses to ecological stressors (Hau et al., 2016; Crossin et al., 2016). However, a study in the common lizard *Lacerta vivipara* showed that daily elevations of corticosterone, a primary glucocorticoid in vertebrates, significantly influenced food consumption and activity levels, and enhanced male survival (Cote et al., 2006). In the same species, daily maternal glucocorticoid elevations resulted in male offspring with higher survival (Meylan and Clobert, 2005). Together, these results indicate a likely mediating effect of short-term elevations of glucocorticoids on fitness that should be further explored.

Here, we test the hypothesis that ecologically relevant changes in glucocorticoid levels directly affect fitness. Specifically, we examined the effects of frequent (daily), short-term increases (to ecologically relevant levels) in glucocorticoids in gravid, eastern fence lizards, *Sceloporus undulatus*. These lizards are subject to a variety of stressors in their environment, the physiological effects of which have been well-studied, allowing us to mimic natural glucocorticoid responses to stressors under controlled laboratory conditions (e.g., Trompeter and Langkilde, 2011; McCormick et al., 2017). Previous work has linked glucocorticoids to increased survival in males (Cote et al., 2006); however, given the increased energetic demands imposed by reproduction (Crespi et al., 2013), and negative effects of environmental stressors on reproductive female survival in other species (MacLeod et al., 2018), we predicted that elevated glucocorticoids during gestation should result in decreased adult female survival and reduced reproductive output in terms of offspring hatching success.

## 2. Materials and methods

### 2.1. Study species, capture, and housing

The eastern fence lizard is a small, diurnal lizard found across the south-eastern portion of the United States (Powell et al., 2016). The glucocorticoid response of this species to a number of environmental stressors has been well-studied, including social stressors (Klukowski and Nelson, 1998; Smith and John-Alder, 1999), and to encounters with a key predator, the invasive red imported fire ant (*Solenopsis invicta*), with which the eastern fence lizard coincides across the southern part of their range (Graham et al., 2012; Langkilde, 2009a). Other physiological responses to ecological stressors have also been shown in this species (Angilletta et al., 2013; Levy et al., 2015). We captured female *S. undulatus* from three populations in southern Alabama (Geneva State Forest, Blakeley State Park, and Conecuh National Forest) in April and May of 2015 and 2016. Upon capture, we measured mass (to nearest 0.01 g) and snout-vent length (SVL, mm), and blood samples were collected from the postorbital sinus. Mean interaction time (from first seeing the lizard to completing blood sampling) was 241.5 ± 141.5 s (mean ± 1 s.e.; approximately 4 min). Gravidity was determined by abdominal palpation upon capture (Graham et al., 2012) and non-gravid females were released.

Gravid females were housed in opposite-treatment pairs (CORT and vehicle-control) in plastic tubs (46 × 40 × 30 cm L × W × H) in a temperature-controlled room (21 ± 1 °C) from capture until laying (a mean duration of 35.5 ± 16.7 days). Tubs contained plastic perches and shelters, water bowls, and moist sand in which females could lay eggs. Heat was provided by a 60-W incandescent light bulb suspended over one end of each tub for 8 h a day to maintain a daytime temperature of approximately 32 °C, with the cooler end of the tub maintaining a temperature of approximately 21 °C, allowing lizards to behaviourally thermoregulate. Overhead lights were maintained on a 12:12 light:dark schedule (0700–1900 h). Food (live crickets, *Acheta domestica*, dusted twice weekly with calcium, vitamins, and minerals) was provided every other day, and water was available *ad libitum*.

### 2.2. Experimental glucocorticoid treatment

Gravid females were randomly assigned to either the control or experimental treatment group. Groups were made identifiable by placing a small dot of color-coded nail polish (free of formaldehyde, toluene and dibutyl phthalate; Pure Ice, New York, NY) on the lizards' backs. From capture until laying, females in the experimental group received a daily transdermal application of a corticosterone solution (hereafter CORT, the primary glucocorticoid in reptiles; Meylan and Clobert, 2005), corrected for their body weight (0.2 μL/g lizard of 4 mg CORT [≥92%, Sigma C2505, Saint Louis, MO] in 1 mL of sesame seed oil vehicle, resulting in doses of 0.8 μg CORT/g body mass). Females in the control group received a dose of the sesame seed oil vehicle only. CORT or control solutions were applied with a pipette to the middle of their back between 1930 and 2030 h. This timeframe was chosen as this was during the lizards' resting period, minimizing disturbance by researchers. There is no evidence of strong diel patterns in CORT secretion in this species (Trompeter and Langkilde, 2011). Handling the lizards was not required for this treatment. A time-series experiment showed that this dosage results in a short-term increase in CORT, doubling baseline levels by 30 min post-dosing (mean baseline CORT 9.97 ng/ml ± 1.81, mean 30 min post-dosing CORT 21.77 ng/ml ± 4.37), with CORT returning to baseline levels by 90 min post-dosing (mean 90 min post-dosing CORT 11.80 ng/ml ± 1.29; see Appendix S1). Note that there is likely to be variation between individuals in the time and level of the CORT peak post-dosing.

This procedure and dosage has been shown in prior experiments to successfully mimic the increase in plasma CORT after non-lethal exposure to fire ants (McCormick et al., 2017; Owen et al., 2018), as well as periods of heat stress (R. Telemeco, pers. comm.). Similar levels of CORT are also achieved by employing a chasing (Trompeter and Langkilde, 2011) and restraint stressor (Graham et al., 2012), suggesting that this level of CORT-increase approximates the glucocorticoid response to short term, ecologically-relevant stressors in this species rather than reflecting pharmacological levels. Thus, as well as being relatively non-invasive (allowing us to avoid any potential confounds with handling stress, stress of dosing by injection, etc.) this treatment allows us to mimic the short-term increase in CORT experienced by free-living lizards who encounter natural stressors daily, rather than the sustained release of hormone implants (e.g. Breuner et al., 2008; Crossin et al., 2016) or pharmacologically high levels (Boonstra, 2013).

Tubs were checked multiple times daily for signs of egg-laying (freshly dug sand, the presence of nesting holes, mass loss/shape change in females). Upon laying, eggs were immediately transferred to plastic containers filled with moist vermiculite (−200 kpa) and sealed with plastic wrap. Containers were kept in temperature-controlled incubators (30 ± 1 °C), and rotated regularly to avoid position effects, until hatching (after approximately 45 days). This protocol has been successfully employed as part of a number of studies (e.g. Langkilde and Freidenfelds, 2010; Swierk and Langkilde, 2013; Trompeter and Langkilde, 2011), with hatching success (proportion clutch hatched)

of > 80% (Swierk and Langkilde, 2013). Females were weighed immediately after discovery of eggs in order to obtain post-partum mass (to nearest 0.01 g).

Over the two years this study was conducted, 78 females were included in this experiment for which we have all relevant measures: 39 females from 2015: 22 control and 17 CORT-treated; 39 females from 2016: 21 control and 18 CORT-treated. Treatment groups did not differ in plasma CORT levels ($X^2_{1,78} = 0.08$, $P = 0.78$) or body condition ($X^2_{1,78} = 0.002$, $P = 0.96$) at capture. Similarly, there were no between-year differences in plasma CORT ($X^2_{1,78} = 0.01$, $P = 0.92$) and body condition ($X^2_{1,78} = 1.83$, $P = 0.18$) at capture.

### 2.3. Statistical analysis

All statistics were conducted using the R software (version 3.2.3; R Core Team, 2015). Female mortality during the experiment (from first capture to 2 weeks after laying) was recorded as a binary variable (0 = survived/1 = died). Clutch success or failure (whether some proportion of eggs hatched – "success" – or all eggs failed – "failure") was recorded as a binary variable (0/1). Proportion hatch success was calculated as the number of eggs that successfully hatched from each clutch (successes) and the number of eggs that did not hatch from each clutch (failures) in a bound column. This binomial dependent variable therefore also accounts for variation in clutch size. Effects of maternal treatment on maternal survival, the likelihood of hatch success/failure, proportion within-clutch hatching success, and female body condition post-laying (residuals of correlation between log-transformed mass and SVL), were tested using generalized linear mixed models (GLMMs), with treatment (control, CORT) and year (2015, 2016) set as explanatory fixed effects. Post-laying mass was not possible to obtain from females that died before laying; we therefore only were able to test treatment effects on post-treatment body condition in surviving females. Binomial error structures were used when appropriate (models of maternal survival and hatch success/failure). An interaction term treatment * year was included in analyses of survival, female body condition post-laying, and hatching success, to assess differences in treatment effects between years. Where interaction terms were significant, $P$ values for individual fixed effects are not reported, as statistical significance is likely to be confounded by the interaction in models. Maternal condition at capture, and number of days of treatment (either CORT or control), were also included as covariates in models of maternal survival to account for variation in initial maternal condition, and variation in opportunity (time) to die/dose number (days of treatment = number of doses, as doses were administered daily). The number of days females were treated, and maternal condition at capture, were included in models of hatch success. All models included site of maternal origin as a random term to account for potential non-independence of multiple individuals from the same sites.

### 2.4. Ethics approval

The research adhered to the Guidelines for the Use of Animals in Research and the Institutional Guidelines of Penn State University (IACUC #44595), and animal collection was permitted by the Alabama Department of Conservation and Natural Resources.

## 3. Results

### 3.1. Maternal survival

We found significant effects of CORT treatment ($Z_{74} = 3.44$, $P < 0.001$) on female survival (Fig. 1a). CORT-treated females had reduced survival; a total of 18 females died over the two years, 15 of which were in the CORT-treated group. Survival was generally lower in 2016 than in 2015 ($Z_{74} = 2.70$, $P < 0.01$), but the effects of treatment were the same in both years tested (treatment * year $Z_{70} = 0.78$, $P = 0.45$). Neither body condition at capture ($Z_{74} = -0.29$, $P = 0.77$), nor number of days in treatment ($Z_{73} = 1.58$, $P = 0.11$; treatment * treatment days $Z_{70} = -1.16$, $P = 0.23$), were important in predicting female survival.

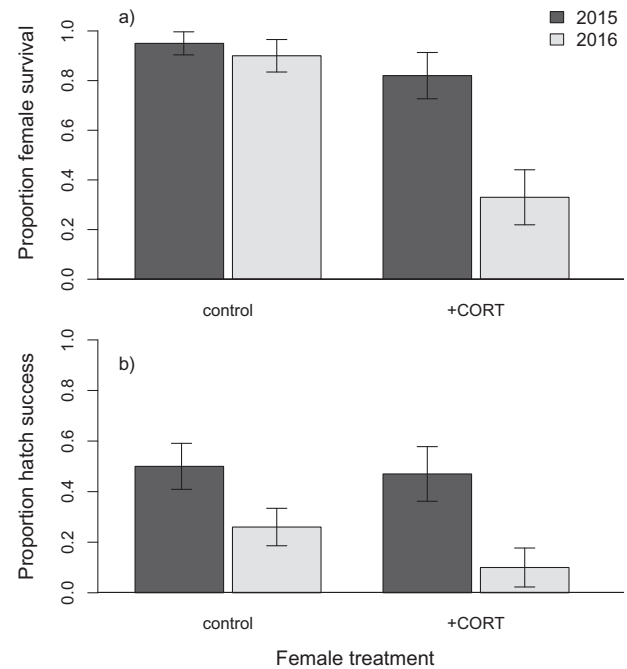


**Fig. 1.** Effects of daily transdermal CORT treatment during gestation on a) proportion of *Sceloporus undulatus* maternal survival and b) proportion hatching success of clutches laid (eggs hatched/total number of eggs laid). Bars represents means ± one standard error.

### 3.2. Adult female body condition

We found that neither CORT-treatment ($T_{52} = 1.09$, $P = 0.27$) nor year ($T_{53} = -1.42$, $P = 0.15$) affected female post-laying body condition. Treatment effects on post-laying condition did not differ between years (treatment * year $T_{50} = -0.63$, $P = 0.49$). Length of time in treatment did not predict post-laying body condition ($T_{51} = -1.10$, $P = 0.25$), and treatment effects did not differ according to length of treatment (treatment * treatment days $T_{49} = -0.81$, $P = 0.39$).

### 3.3. Hatching success

There was an interactive effect of treatment and year on hatching success (treatment * year $Z_{45} = -3.86$, $P < 0.001$; treatment $Z_{45} = 4.01$; year $Z_{45} = -4.42$; Fig. 1b). Clutches laid by CORT-treated females had lower proportional hatching success (number of eggs that viably hatched/total number of eggs laid), and this effect was greater in 2016 than in 2015. Further, CORT-treated female's hatching success decreased the longer they were treated, whereas control female's hatching success increased the longer they were treated with the vehicle (treatment * treatment length $Z_{45} = -3.87$, $P < 0.001$). Female body condition at capture did not influence hatching success of their clutches ($Z_{44} = 0.34$, $P = 0.34$).

### 3.4. Year effects

Compared to 2015, we found that in 2016: i) our treatment effects on hatching success were stronger, and ii) adult female survival was reduced overall. These differences may have resulted from a significantly warmer 2015/2016 winter (November – April, prior to the 2016 sampling) compared to 2014/2015 (Fig. 2; see Appendix S2 for

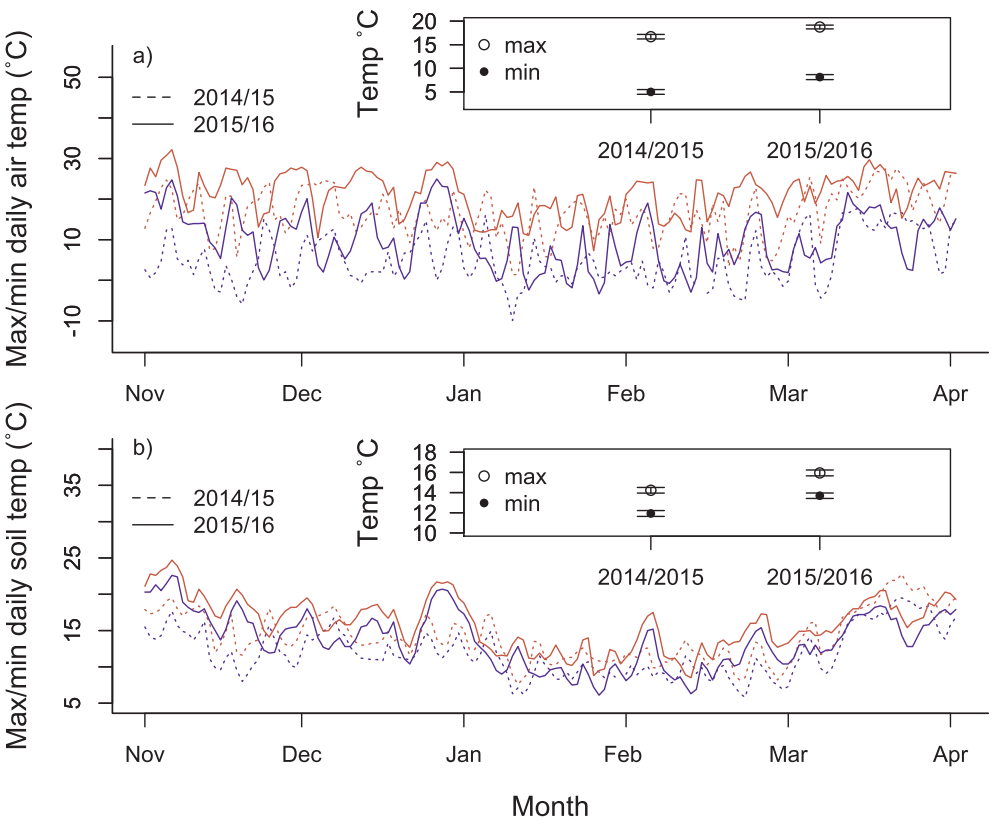


**Fig. 2.** Daily maximum (red, top line) and minimum (blue, bottom line) a) air and b) soil (at a depth of 4in) temperatures between 1st November and 1st April in 2014/15 (dashed lines) and 2015/16 (solid lines). Inset shows the average daily minimum and maximum temperatures for these two time periods, ± one standard error. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

details). Comparison (using $T$ tests) of the daily maximum and minimum ambient temperatures showed that temperatures were higher in the over-winter period in 2015/2016 than in the same period in 2014/2015 (daily max $T_{290} = -3.35$, $P < 0.001$; daily min $T_{298} = -4.29$, $P < 0.0001$). These patterns were the same for daily minimum and maximum soil temperatures (at a depth of 4 in.) ($T$ tests: daily max $T_{299} = -4.29$, $P < 0.0001$; daily min $T_{293} = -4.30$, $P < 0.0001$) (National Water and Climate Center, 2017). This warmer winter was associated with all captured females (49 from 2015, 57 from 2016) showing a trend for lower body condition (residuals of weight on size $F_{2,97} = 3.05$, $P = 0.08$) and lower blood glucose ($T_{30} = 2.95$, $P < 0.01$) at capture in 2016 than in 2015 (all measures accounting for gravidity).

## 4. Discussion

We demonstrate a causal link between repeated glucocorticoid exposure at ecologically-relevant levels and reduced maternal and egg survival in a lizard, *Sceloporus undulatus*. Experimental evidence of the effects of repeated short-term glucocorticoid elevation on fitness-related traits remains relatively scarce in wild animals (Breuner et al., 2008). Short-term elevations of glucocorticoids as part of the stress response promote immediate survival and therefore are assumed to be adaptive (Wingfield et al., 1998); for example, in the eastern fence lizard, short-term elevations in circulating glucocorticoids have been shown to promote anti-predator behaviors in the wild that maximize immediate survival (Langkilde, 2009a,b; Trompeter and Langkilde, 2011). However, we show that, although this physiological response to environmental stressors is likely to be immediately beneficial, if continuously repeated over longer times scales (weeks), it may come at a cost to fitness. Fitness costs of physiologically-relevant increases in glucocorticoids have previously been considered to be unlikely in natural systems (Boonstra, 2013). By testing the effects of glucocorticoid elevations in isolation we are able to remove confounding effects of other aspects of the physiological stress response via the HPA axis or the stimulus itself (e.g. fire ant venom). Effects of maternal exposure to stressors may therefore differ depending on the nature of the stressor, as factors other than glucocorticoids may be playing a role. However, our findings provide some support for glucocorticoids being a possible mechanism by which environmental stressors such as predation risk can reduce organismal fitness in wild populations (MacLeod et al., 2018; Sheriff et al., 2009).

Although frequent elevations of glucocorticoids have been shown to have negative effects on body condition in Gambel's white-crowned sparrow (*Zonotrichia leucophrys gambelii*) (Busch et al., 2008), and positive direct or indirect effects on survival in common lizards (*Lacerta vivipara*), to our knowledge our results provide the first evidence that frequent, short-term glucocorticoid elevation within ecologically-natural ranges can *reduce* survival. The reduced survival we show is unlikely to have been caused by ecologically improbable pharmacological consequences resulting from our experimental treatment, given that we have previously shown that CORT dosages applied are within ecologically-relevant levels and return to baseline levels 90 min post-dose. We suggest our experimental treatment increased the potential for individuals to reach 'allostatic overload', whereby the energetic demands of the body for maintaining stability in the face of a perturbation (allostasis) exceeds energy income, or the prolonged activation of the physiological systems mediating allostasis leads to pathology (McEwen and Wingfield, 2003; Romero et al., 2009). We were unfortunately unable to test whether females that died were in poorer body condition as might be predicted if these individuals reached allostatic overload, as we sought to minimise handling stress during the experiment and so did not have frequent mass measurements, and mass after death is strongly affected by rapid water loss.

Hatching success was generally low in this study; however, this is not likely to be due to the captive environment or incubation conditions, as previous work in this species using the same incubation protocol in non-CORT related experiments have reported high hatching success (Swierk and Langkilde, 2013). We are not sure of the cause of the low hatching success overall; however, we found that maternal

CORT exposure further reduced survival of offspring at the embryonic stage (within the egg) and that the strength of these effects is related to the length of treatment. Although there is growing evidence that maternal exposure to environmental stressors can reduce reproductive output (e.g., predation risk affects clutch size (Eggers et al., 2006; Travers et al., 2010) and reduces offspring survival both pre- and post-natally (MacLeod et al., 2018; Zanette et al., 2011)), the evidence for glucocorticoids as a mechanism for such effects is scarcer (Breuner et al., 2008; but see Sheriff et al., 2009). In Atlantic salmon (*Salmo salar*), experimentally elevated maternal glucocorticoid levels reduced egg survival, potentially due to reduced embryo yolk sac volume (Eriksen et al., 2006). Similarly, in a viviparous gecko (*Hoplodactylus maculatus*), experimentally elevated maternal glucocorticoid levels (via implants) increased the likelihood of developmental abnormalities and abortion, and reduced embryonic growth (Cree et al., 2003). Although it seems likely that an increase in maternal glucocorticoids has the potential to reduce embryonic survival, it has been suggested that surviving offspring may be better adapted to their future environment (Sheriff et al., 2017). For example, Meylan and Clobert (2005) demonstrated that elevated maternal glucocorticoids increased juvenile survival in common lizards (*Lacerta vivipara*). Future studies investigating the long-term survival and reproductive success of surviving offspring would therefore be an informative next step in determining the effects of maternal glucocorticoid effects on offspring fitness.

Our results are in contrast to findings in the common lizard, *Lacerta vivipara*, which demonstrated *increases* in male survival following short-term corticosterone elevations (Cote et al., 2006; Meylan and Clobert, 2005). The risk of reaching allostatic overload can be especially great during reproduction, when the energetic demands and glucocorticoid levels of gravid females are greatly increased (Crespi et al., 2013) bringing females naturally closer to their allostatic overload threshold. This could explain why we show reductions in survival while other studies have shown increases in survival in males: gravid females are under the additional stress of reproduction, which may amplify effects of CORT.

The burden of multiple stressors may also explain why we saw strong between-year differences in effects of maternal glucocorticoid treatment. Glucocorticoid effects on survival may only manifest when organisms are already challenged, for example, in the presence of an environmental stressor (Sopinka et al., 2015). For example, cortisol-treated largemouth bass succumbed more rapidly to harsh over-winter conditions than control fish (O'Connor et al., 2010). Our findings tentatively suggest that periods of climatic stress, such as winter warming, may amplify the effects of subsequent stressors animals face and result in greater fitness costs. It has previously been shown that hibernating ectotherms, such as eastern fence lizards (John-Alder et al., 2009), have higher metabolic rates and energy demands as a consequence of warmer over-winter temperatures, which can lead to reduced body condition in Spring when breeding occurs (Brischoux et al., 2016; Muir et al., 2013), which may increase susceptibility to CORT effects. Similar decreases in survival of offspring from populations subject to high levels of fire ant predation (in which baseline CORT levels are higher, Graham et al., 2012) have been shown following years of drought and warm winters, further supporting this premise (Owen et al., 2018). It is possible that other factors differed between these two years in addition to weather, including random cohort differences. Our results suggest that further research examining the influence of environmental context, particularly overwinter conditions, on the effects of glucocorticoid elevation would be fruitful.

We show that frequent elevation of glucocorticoids at ecologically-relevant levels can reduce both survival and reproductive success in a wild animal. Thus, although a short-term increase in glucocorticoids may increase immediate survival in response to a stressor, we directly demonstrate the consequences of repeated increases in glucocorticoids. We also provide tentative evidence that a previously experienced climatic stressor, warmer winters, can increase animal vulnerability and amplify the effects of the current stressor they face. As animals are exposed to human-induced environmental change, they will face increasing and novel stressors, such as invasive species and climatic warming. Our results show that we must examine the interactive effects across both space and time to better understand how glucocorticoid elevations can influence the fitness and population health of wild animals.

## Data availability

Data used in this study are deposited at DOI: http://dx.doi.org/10.5061/dryad.p5n6g86.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ygcen.2018.07.006.

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