

Survival is negatively associated with glucocorticoids in a wild ungulate neonate

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Abstract

It is unknown how ungulate physiological responses to environmental perturbation influence overall population demographics. Moreover, neonatal physiological responses remain poorly studied despite the importance of neonatal survival to population growth. Glucocorticoid (GC) hormones potentially facilitate critical physiological and behavioral responses to environmental perturbations. However, elevated GC concentrations over time may compromise body condition and indirectly reduce survival. We evaluated baseline salivary cortisol (CORT; a primary GC in mammals) concentrations in 19 wild neonatal white-tailed deer (*Odocoileus virginianus*) in a northern (NS) and southern (SS) area in Pennsylvania. After ranking survival models consisting of variables hypothesized to influence neonate survival (i.e. weight, sex), the probability of neonate survival was best explained by CORT concentrations, where elevated CORT concentrations were associated with reduced survival probability to 12 weeks of age. Cortisol concentrations were greater in the SS where predation rates and predator densities were lower. As the first evaluation of baseline CORT concentrations in an ungulate neonate to our knowledge, this is also the first study to demonstrate CORT concentrations are negatively associated with ungulate survival at any life stage. Glucocorticoid hormones could provide a framework in which to better understand susceptibility to mortality in neonatal white-tailed deer.

Key words: neonates, salivary cortisol, stress, survival, ungulates

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INTRODUCTION

Physiological reactions are among an organism's first responses to environmental perturbations (Sapolsky 2002) and can indicate how organisms are influenced by environmental variables (Ditmer *et al.* 2015). Touted as conservation-relevant biomarkers, physiological measurements may provide a holistic understanding of organismal well-being and mortality risk in diverse

environments (Edwards *et al.* 2019). Much of the current understanding of physiological responses to ecological stressors; however, results from laboratory and experimental studies with tightly controlled settings (Sopinka *et al.* 2015). Evaluating the physiology of wild populations in their natural habitat may provide a more representative understanding of how environmental conditions influence wildlife populations.

Glucocorticoids (GCs, e.g. corticosterone and cortisol) are steroid hormones released by the hypothalamic-pituitary-adrenal (HPA) axis as one part of a complex neuroendocrine response following HPA axis activation (Sapolsky 2002). Activation of the HPA axis, and subsequent GC release, occurs in response to a variety of stimuli (e.g. weather, food supply, presence of conspecifics) and integrates an organism's response to environmental stimuli into a measurable physiological trait (Wingfield 2013; Ditmer *et al.* 2015). Glucocorticoid release can influence many individual characteristics including survival (Brown *et al.* 2005; MacLeod *et al.* 2018), but whether GC release is adaptive or potentially detrimental to an individual's survival may depend on the magnitude and duration of GC release (Cabezas *et al.* 2007). For example, short-term elevated GC concentrations can be adaptive, allowing quick responses to immediate stressors, thereby increasing survival (Sapolsky *et al.* 2000). Alternately, chronically elevated baseline concentrations can negatively influence an individual's health and indirectly decrease survival if energy is allocated to ameliorating stressors at the expense of self-maintenance (McEwen & Wingfield 2003; Romero 2004).

Numerous and varied mechanisms may reduce survival in individuals with elevated baseline glucocorticoids (Bonier *et al.* 2009) because GCs can alter the properties and function of many cell types (Hau *et al.* 2016) such as neurons (Lupien *et al.* 2009) and proteins (Black 1994) that are potentially important for survival. For example, GCs can promote the conversion of protein and lipids into usable carbohydrates (McEwen & Wingfield 2003) while increasing metabolism rates (Haase *et al.* 2016) and influencing brain functions that can result in elevated energy expenditure via modified behaviors (e.g. increased locomotor activity; Cote *et al.* 2006). Chronically high GC concentrations, however, may result in prolonged periods of energy expenditure that can reduce body weight (Cabezas *et al.* 2007) and individuals with reduced body weight may experience greater susceptibility to mortality (Vreeland *et al.* 2004). Immune system responses can also be influenced by GCs (Black 1994; Sopinka *et al.* 2015) with potential consequences for survival (Nelson *et al.* 2002). Although there are a variety of mechanisms by

which elevated GCs can impair immune responses (Black 1994; Sopinka *et al.* 2015), one such mechanism suggests GCs reduce the production of cytokine proteins that help mediate inflammation and may explain why juvenile rats experimentally exposed to elevated GC experienced increased tumor growth (Black 1994). Although elevated GCs can indirectly cause reduced survival (Ouyang *et al.* 2015; MacLeod *et al.* 2018), the relationship between survival and GCs is not consistent (Brown *et al.* 2005; Cabezas *et al.* 2007; Romero & Wikelski 2010). Complex mechanisms linking survival and GCs may help explain why the relationship remains largely unexplored (Cabezas *et al.* 2007), with little literature experimentally or observationally describing such links or mechanisms in large, wild mammals and especially their neonates.

Although wild ungulates are some of the most socio-economically important species in North America (Huijser *et al.* 2009), whether elevated GC concentrations influence ungulate population demographics is largely unstudied (Creel *et al.* 2013). Although elevated GC concentrations in adult ungulates have been associated with various factors such as ambient temperature (Huber *et al.* 2003), age (Santos *et al.* 2018), and human disturbance (Creel *et al.* 2002; Ensminger *et al.* 2020), it is uncertain if the elevated GC concentrations influenced individual fitness or population demographics such as survival and reproductive rates. However, failing to detect factors influencing demographics may undermine efforts aimed at anticipating demographic changes and identifying appropriate management responses. Studies designed to investigate the link between GC responses and population demographics are needed across ungulate life history stages to fully elucidate how GCs may influence population demographics (Creel *et al.* 2013).

As a primary component determining population growth (Gaillard *et al.* 2000; Raitzel *et al.* 2007), ungulate neonate mortality is well studied by conservationists and wildlife managers (Linnell *et al.* 1995; Gingery *et al.* 2018). However, only one record of GC concentrations in wild, ungulate neonates is currently published and describes unexpectedly high acute GC responses to handling events in neonatal roe deer (*Capreolus capreolus*; Bonnot *et al.* 2018). Evaluating factors that elevate neonate GC concentrations, and subsequent effects on individual fitness, may be important. Studies conducted in other mammal species indicate GC exposure early in life can result in life-long reduction in GC responses to stressful stimuli (Caldji *et al.* 2010), delayed puberty (Macfarland & Mann 1977) and weight loss (Lopucki *et al.* 2019). Whereas acute GC responses may be greater

than previously thought, nothing is known about ungulate neonatal baseline GC concentrations.

White-tailed deer (*Odocoileus virginianus*) are one of the most important ungulate species in North America (Conover 1997; U.S. Fish and Wildlife Service 2011), and their abundance can influence plant and animal ecological communities (Rooney & Waller 2003; Nuttle *et al.* 2011) and cause economic loss (Conover & Kania 1995; Côté *et al.* 2004; Bissonette *et al.* 2008). In Pennsylvania, white-tailed deer neonate (hereafter neonate) survival differs between two populations located in north-central (0.51) and central Pennsylvania (0.71; Gingery *et al.* 2018), which may be related to differences in predator abundance (Vreeland *et al.* 2004). It is unknown if factors such as GC concentrations may be associated with variable neonate mortality in Pennsylvania.

Here, we hypothesize that elevated baseline salivary cortisol (hereafter CORT, the primary GC in mammals) concentrations will be associated with reduced probability of survival to 12 weeks of age in wild, neonatal white-tailed deer. Because it is unclear whether the factors that elevate adult ungulate GC concentrations also elevate neonate baseline CORT concentrations, we also investigated associations between ecological variables and CORT concentrations. We hypothesized that the area where a neonate was captured (e.g. northern versus southern Pennsylvania) would be the strongest predictor of neonate CORT concentrations compared to other ecological variables (e.g. temperature, weight). Because predators are the primary source of mortality for white-tailed deer neonates (including the populations in our study; Gingery *et al.* 2018), we predicted CORT concentrations would be greatest in the northern study area where predator density, neonate mortality, and predation rates are greater (Ternent 2016; Gingery *et al.* 2018).

MATERIALS AND METHODS

Study sites and sample collection

We captured deer in 2 study areas in Pennsylvania. The northern study area (NS, 155 km²) was located in the Susquehannock State Forest in Potter County which is part of the Deep Valleys section of the Appalachian Plateaus physiographic region (Cuff *et al.* 1989). In the region surrounding the NS, the Northwest Plateau, the mean annual temperature was 8.6°C and mean annual precipitation was 1.16 m (NRCC 2018). The NS topographic features were plateaus at approximately 800 m elevation dissected by steep drainages dropping to approximately 220 m. A mixture of northern hardwoods and

conifer forest types comprised 88% of the NS. Dominant tree species were red maple (*Acer rubrum*), sugar maple (*Acer saccharum*), black cherry (*Prunus serotina*), and American beech (*Fagus grandifolia*). Agricultural land cover (row crops, hay and alfalfa fields, pastures) comprised <1% of the total land area. The remaining land cover was open water, fallow ground, barren land, or open space. In 2015, pre-hunting season deer densities in the NS were 9.2 deer/km² (Rosenberry *et al.* 2016). Potential predator species included black bear (*Ursus americanus*), coyote (*Canis latrans*), red fox (*Vulpes vulpes*), and bobcat (*Lynx rufus*). Adult female deer survival in the NS was high, ranging from 0.990 (SE ± 0.007) to 0.997 (SE ± 0.003) from May to August.

The southern study area (SS, 239 km²) was located in Centre, Mifflin, and Huntingdon Counties and is in the Ridge and Valley physiographic region in central Pennsylvania (Cuff *et al.* 1989). The mean annual temperature was 8.9°C and mean annual precipitation was 1.14 m (NRCC 2018). The SS contained 178 km² of Rothrock State Forest and approximately 6 km² of Bald Eagle State Forest. Topographic features consisted of long, parallel ridges and valleys along a northeast-southwest axis where elevation was 400 to 700 m above sea level (Cuff *et al.* 1989). Agricultural land cover (row crops, hay, alfalfa, and pastures) comprised 15% of the total SS land area. The oak-hickory forests contained an understory layer of ericaceous shrub species (*Vaccinium* spp., *Gaylussacia* spp., *Gaultheria procumbens*, and *Kalmia latifolia*) and comprised 79% of the SS land area. Dominant tree species were red and white oak (*Quercus* spp.), red maple, black birch (*Betula lenta*), black gum (*Nyssa sylvatica*) and hickory (*Carya* spp.). In 2015, pre-hunt deer densities in the SS were approximately 10.3 deer/km² (Rosenberry *et al.* 2016). Predation rates were greater in the NS than the SS (Gingery *et al.* 2018) where harvest and population data indicated predator densities were higher in NS than in SS (Rosenberry 2009; Johnson 2016). Potential predator species included black bear, coyote, red fox, and bobcat. Adult female deer survival in the SS was high, ranging from 0.987 (SE ± 0.009) to 0.996 (SE ± 0.004) from May to August.

We captured adult female deer using rocket nets and single-gate clover traps (Hawkins *et al.* 1968) from January to April 2017 to facilitate neonate capture by inserting vaginal implant transmitters (VITs). We sedated adult females with a 150 mg intramuscular injection (IM) of xylazine hydrochloride and subsequently inserted a VIT and fitted each deer with a GPS radiocollar (GPS Plus, Vectronic Aerospace, Berlin, Germany). We reversed sedation with 150 mg tolazoline hydrochloride given IM.

All protocols were approved by The Pennsylvania State University Institutional and Animal Care and Use Committee (Protocol No. 47054). Following adult captures, we linked VITs and GPS radiocollars remotely. From May to July when the VITs were expelled, the GPS collar transmitted a message that a potential birth had occurred. We waited at least 6 hours after receiving notification of a potential birthing event before initiating searches to allow maternal-offspring social bonding (Kilgo *et al.* 2012). If no neonates were located during the initial search, we conducted a second search waiting at least 6 h between searches.

We caught neonates from May to June in 2017 using expelled VITs and opportunistic searches. During opportunistic neonate capture attempts, we drove vehicles along roads and trails while provoking postpartum maternal behaviors in female deer with a fawn bleat to help indicate whether a female had a neonate nearby (Diem 1954; White *et al.* 1972; Vreeland *et al.* 2004). Upon observing maternal behaviors of isolated does, a team of 2 to 5 people conducted a grid search of the immediate area. Field crews in the SS also searched hay and alfalfa fields located on private and state forest lands in addition to searches by vehicle. Hay and alfalfa fields were not present in the NS.

Once located, we restrained and blindfolded neonates and documented the time that first contact was made. After blindfolding neonates, we immediately began collecting saliva samples to ensure baseline stress sampling, as salivary CORT concentrations increase 20–30 min after the onset of a stressor (Millsbaugh *et al.* 2002). To collect saliva samples, we placed 3–6 cotton swabs with a long plastic shaft in the mouth of fawns and left swabs in place until saturated. If needed, we left cotton swabs in place while we documented neonate sex, capture location, and site characteristics, and fitted fawns with an expandable, very high frequency (VHF) radiocollar (Vectronic Aerospace or Advanced Telemetry Systems, Isanti, Minnesota, USA; Diefenbach *et al.* 2003). We did not collect saliva if it took ≥ 20 min to saturate cotton swabs (Millsbaugh *et al.* 2002). As a result, CORT concentrations reflected baseline concentrations prior to the capture disturbance and not the subsequent increase in CORT concentrations in response to capture. We removed cotton swabs after saturation and placed each swab upside down in a plastic vial (Millsbaugh *et al.* 2001). We ear-tagged fawns with a numbered, plastic tag in each ear (National Band and Tag, Newport, Kentucky) imprinted with a toll-free telephone number to collect a \$100 reward if found. We released fawns where they were captured.

After neonate release, we immediately centrifuged vials containing cotton swabs. We centrifuged vials at 10 000 rpm to fully separate the cotton and saliva, resulting in pooled saliva from each individual swab at the bottom of the vial (Millsbaugh *et al.* 2001). We removed the cotton swabs after centrifugation and froze samples at -20°C until they could be transported to Pennsylvania State University for analysis.

We monitored the survival of radio-collared fawns via ground-based telemetry twice daily from capture until mid-August, 1 to 7 times weekly thereafter until mortality, collar failure, or the end of the study. Collar transmitters remaining motionless for 4 h transmitted a signal indicating that a possible mortality occurred. We investigated all fawn mortalities within 24 h of signal detection.

Saliva sample processing

We quantified salivary cortisol using a high-sensitivity enzyme immunoassay kit, following manufacturer instructions (kit 1–3002; Salimetrics, State College, PA, USA). Quantifying salivary cortisol using high-sensitivity enzyme immunoassay kits has been validated in white-tailed deer (Millsbaugh *et al.* 2002). We analyzed all samples in duplicate, and we excluded samples in which the coefficient of variation was $\geq 10\%$ between duplicates. Assay sensitivity was $< 0.007 \mu\text{g/dL}$ and intra-assay CV was 9.14, where all samples were run on the same plate.

Data analysis—modeling CORT concentrations

To evaluate the factors that may be associated with neonate CORT concentrations, we developed 9 a priori candidate linear models where CORT concentration was the response variable in Program R (R Development Core Team, R version 3.6.2). Predictor variables were those that may be associated with CORT (ambient temperature on capture day, sex, distance to road, percent forested within 200 m of capture location, body mass; Table 1). Also, we evaluated the time of day at which the sample was collected to account for diel variation (Breuner *et al.* 1999) and time elapsed (sampling time–start time) to evaluate the assumption that we were not yet measuring the effects of handling (Romero & Reed 2005). We used QQ plots and plots of regression residuals to evaluate the assumptions of linear models and subsequently log-transformed the response variable (CORT concentrations) to meet assumptions of normally distributed residuals and variance.

To identify the model with the most empirical support (i.e. the model that best explained the CORT concentrations we observed), we used Akaike's Information

Table 1 Model selection statistics for 9 linear models estimating white-tailed deer (*Odocoileus virginianus*) neonate salivary cortisol (CORT) concentrations following birth in north-central (NS) and central (SS) Pennsylvania, 2017

Model	Model description	ΔAIC_c^\dagger	W_i^\ddagger	$-2\log$ - likelihood	k^\S	Intercept (SE)	B_1 (SE)
CORT ~ AREA [¶]	CORT concentrations vary between study areas	0.0	0.53	-19.8	3	-2.88 (0.27)	0.95 (0.37)
CORT ~ Dist	CORT concentrations vary by distance to roads	2.6	0.15	-21.1	3	-2.35 (0.19)	-0.39 (0.20)
CORT ~ Day	CORT concentrations vary by calendar day	2.9	0.12	-21.2	3	-2.35 (0.20)	0.36 (0.20)
CORT ~ Mass	CORT concentrations vary by neonate body mass	4.7	0.05	-22.1	3	-2.35 (0.21)	-0.26 (0.21)
CORT ~ Sex	CORT concentrations vary between sexes	5.5	0.03	-22.5	3	-2.52 (0.28)	0.38 (0.42)
CORT ~ Elapsed	CORT concentrations vary by the amount of time elapsed since contact was made during capture	5.6	0.02	-22.6	3	-2.35 (0.21)	-0.18 (0.22)
CORT ~ Time	CORT concentrations vary by the time elapsed between first disturbance and saliva collection	5.6	0.02	-22.6	3	-2.35 (0.21)	-0.17 (0.22)
CORT ~ Temp	CORT concentrations vary by the temperature on day of capture	6.0	0.02	-22.7	3	-2.35 (0.21)	-0.13 (0.22)
CORT ~ Forest	CORT concentrations vary by the amount of forest cover within 200 m of capture location	6.3	0.01	-22.9	3	-2.35 (0.22)	0.01 (0.22)

Parameter estimates are on the log scale. [†] ΔAIC_c = difference in AIC_c value from the model with the lowest AIC_c value. [‡] AIC_c weight. [§] k = number of parameters. [¶]Categorical. Northern study area (NS) = 0 and southern study area (SS) = 1.

Criterion adjusted for sample size to rank the models (AIC_c ; Burnham & Anderson 2002). Akaike's Information Criterion uses the model likelihood with a penalty term to identify the most parsimonious model among the candidate model set (Burnham & Anderson 2002). Models with the most empirical support receive a ΔAIC_c value of 0.0 and models with a ΔAIC_c value > 2 have less empirical support for the hypothesis that the model represents (Burnham & Anderson 2002). Because predictor variables varied in order of magnitude, all continuous predictors were standardized to mean of zero and variance of one prior to analysis.

Data analysis—modeling survival

We used known-fate models in Program Mark (v. 6.1, White & Burnham 1999), to estimate neonate sur-

vival for the 19 individuals with CORT concentrations. We considered neonates at risk from a common date (i.e. entries were not staggered) to meet the assumption that all individuals had the same mortality risk in each time period (Pollock *et al.* 1989). We censored neonates when a transmitter with no evidence of mortality was recovered.

We developed 9 models of neonate survival where the variable AREA (NS or SS) was combined with individual covariates (Table 2) to account for differences in survival between study sites (Gingery *et al.* 2018). Also, a model was created where survival was only a function of CORT concentration. Individual covariates included CORT concentrations and variables often hypothesized to influence neonate survival such as sex, the number of days a neonate was born from peak fawning (Day), and whether the neonate was the offspring of a

Table 2 Model selection statistics for 9 models describing probability of survival to 12 weeks of age for white-tailed deer neonates (*Odocoileus virginianus*) in 2 study areas, Pennsylvania, 2017

Model	Model description	ΔAIC_c^\dagger	W_i^\ddagger	Model likelihood	-2log-likelihood	k^\S
S (CORT [¶])	Survival varied by CORT concentration	0.0	0.33	1.00	50.72	2
S (AREA + CORT)	Survival varied between study areas and by CORT concentration	0.42	0.27	0.81	49.06	3
S (AREA)	Survival varied between study areas	1.25	0.18	0.53	51.97	2
S (AREA + VIT ^{††})	Survival varied between study areas and whether a neonate was caught with a VIT	2.8	0.08	0.25	51.43	3
S (AREA + Day)	Survival varied between study areas and by the days between capture date and date of peak neonate drop within a year	3.1	0.07	0.21	51.79	3
S (AREA + Sex)	Survival varied between study areas and the sex of the neonate	3.2	0.07	0.20	51.85	3
S (AREA + Week)	Survival varied between study areas and by neonate age in weeks	17.9	0.00	0.00	42.09	14
S (AREA × Week)	Survival varied by week and between study areas	37.8	0.00	0.00	36.67	24
S (AREA × CORT)	Survival varied by CORT concentrations and between study area	51.2	0.00	0.00	101.9	2

[†] ΔAIC_c = difference in AIC_c value from the model with the lowest AIC_c value. [‡] AIC_c weight. [§] k = number of parameters. [¶]Cortisol hormone concentration in saliva at time of capture. ^{††}Vaginal implant transmitter.

female implanted with a VIT. The estimated probability of survival may be lower for neonates captured using VITs due to earlier detection (Gilbert *et al.* 2014). Furthermore, neonates born further from the peak fawning date may be at greater risk of predation (Whittaker & Lindzey 1999). We used June 1st as the statewide peak annual fawn birth date (Diefenbach *et al.* 2019). Models estimated survival to 12 weeks of age because most mortality occurs during the first 12 weeks of life for many ungulate species (Linnell *et al.* 1995; Bonar *et al.* 2016; Eacker *et al.* 2016). We ranked models using AIC_c (Burnham & Anderson 2002).

RESULTS

Salivary CORT concentrations

We collected saliva from 34 neonates. We successfully quantified salivary cortisol concentrations for 19 individuals after samples were discarded that did not provide minimum 25 μ L needed for analysis ($n = 7$) or where

the coefficient of variation was $\geq 10\%$ between duplicates ($n = 8$). Average time between first contact with the neonate and a collected saliva sample was 6 minutes. Cortisol concentrations ranged from 0.02 to 0.68 μ g/dL ($n = 19$, Fig. 1). Eight neonates were male (0.15 μ g/dL, SE \pm 0.08, 4 in NS and 4 in SS) and 11 were females (0.14 \pm 0.03, 4 in NS and 7 in SS). All neonates in the NS ($n = 8$) were captured in forested areas while in the SS ($n = 11$) 6 neonates were caught in agricultural fields (0.19 \pm 0.1) and 5 in forested areas (0.21 \pm 0.07).

Cortisol concentrations were best explained by the study area where the neonate was captured (AREA; Table 1) where CORT concentrations were greater in the SS (0.21 \pm 0.06) than the NS (0.06 \pm 0.01). One neonate did not have a recorded weight; therefore, models were run with the 18 individuals that had both CORT concentrations and weight to include a model with weight as a potential predictor of CORT concentrations. However, the results were unchanged when all 19 individuals were used to evaluate the model set excluding the model that incorporated weight as a predictor variable.

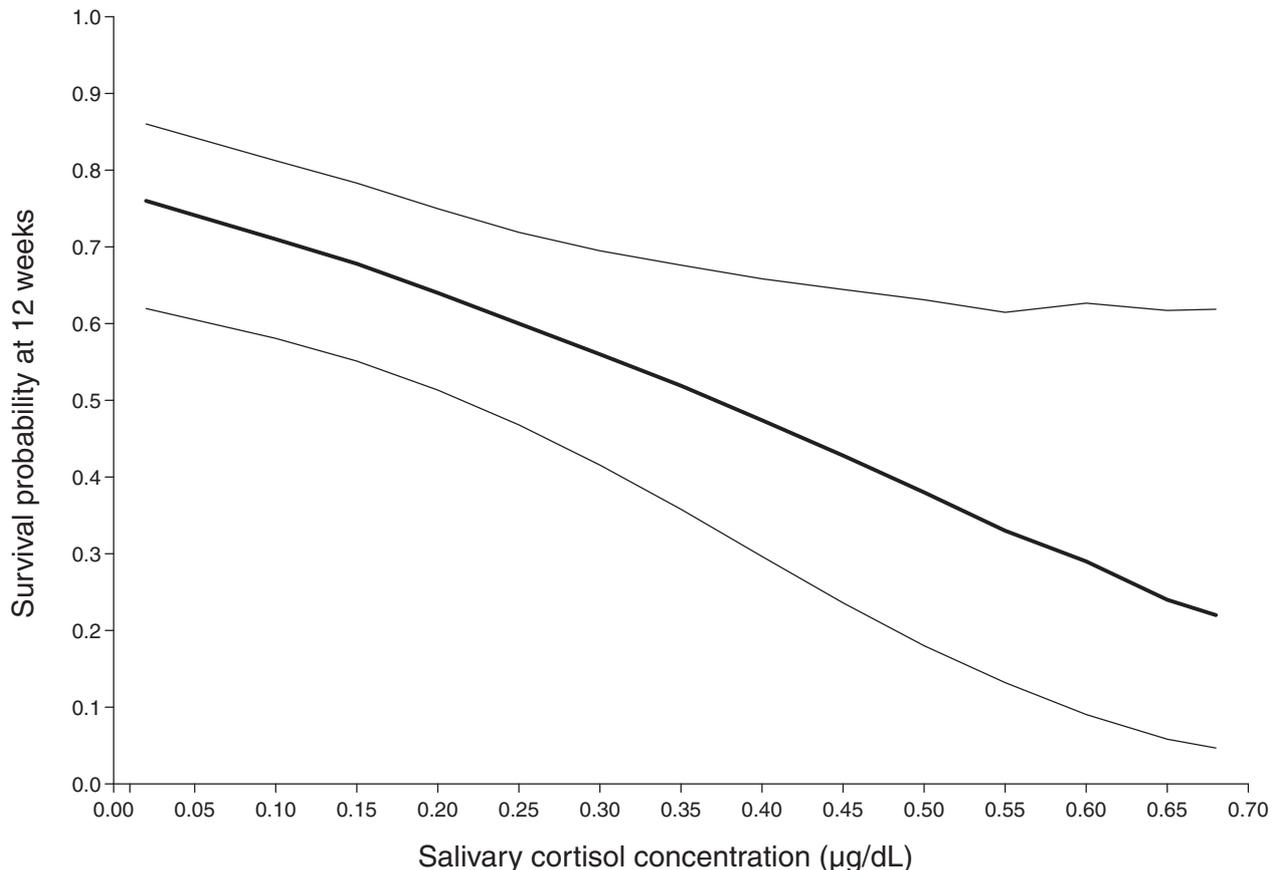


Figure 1 Decreasing probability of survival at 12 weeks of life across the range of observed salivary cortisol concentrations (0.02–0.68 µg/dL) in wild white-tailed deer neonates (*Odocoileus virginianus*, $n = 19$) in Pennsylvania, 2017.

Survival

Of the 19 neonates for which saliva was quantified, 6 neonates died (3 in the NS and 3 in the SS, 3 males and 3 females) where mortality was attributed to predation ($n = 3$), natural (excluding predation; $n = 2$), and unknown causes ($n = 1$). The top model estimating neonate survival indicated CORT concentrations best explained weekly survival to 12 weeks ($n = 19$, Table 2) and that survival was negatively associated with CORT concentrations ($\beta = -2.66$, $SE \pm 1.89$). Probability of survival to 12 weeks of age ranged from 0.76 (0.02 µg/dL) to 0.22 (0.68 µg/dL, Fig. 1).

DISCUSSION

To our knowledge, this is the first study to quantify baseline neonate CORT concentrations and link ungulate neonate GC responses to survival. Because neonate sur-

vival is a primary determinant of population growth rates (Gaillard *et al.* 2000), factors associated with survival such as CORT concentrations may provide insight into ungulate population demographics. Within our limited sample size, we observed decreasing survival probabilities to 12 weeks of age as CORT concentrations increased suggesting CORT concentrations could be a useful metric for evaluating neonate susceptibility to mortality.

Whether reduced neonate survival to 12 weeks was caused by mechanisms resulting from elevated baseline CORT concentrations is unknown. Elevated GCs may cause reduced fitness and survival via suppressed immune function and reduced growth resulting from oxidative stress, altered gene expression, and immunocompetence (see Sopinka *et al.* 2015 for review) where GC-induced oxidative stress could be an underlying mechanism capable of reducing mammalian fitness, with greater influence in juveniles (Costantini *et al.* 2011). Without experimental manipulation, however, we cannot establish

CORT concentrations as the cause of reduced survival that we observed. Because we could not evaluate whether individuals that died were in poorer body condition (or experienced greater oxidative stress) in comparison to those that lived, it remains unknown whether elevated CORT concentrations caused reduced survival or whether unknown environmental factors altered both CORT and survival independently (Taff *et al.* 2018). Rather than causing reduced survival, elevated CORT concentrations may instead reflect a neonate's response to perturbations that reduced survival.

Disentangling the effect of elevated CORT concentrations from other variables capable of influencing survival is difficult, especially in wild populations. Wild neonates are often caught opportunistically without their mother, for example, making it difficult to identify how maternal age, survival, condition, or behaviors influence neonate survival. Lacking the ability to identify or control for any maternal variation, the relationship between neonate survival and CORT concentrations may be confounded because evidence collected in other species suggests maternal GC responses may influence both offspring survival (Gagliano & McCormick 2009; Pinho *et al.* 2019) and CORT concentrations (Wilcoxon & Redei 2007; Ensminger *et al.* 2018) independently.

Regardless of whether elevated CORT concentrations caused reduced survival, we observed CORT concentrations collected at time of capture were associated with reduced neonate survival. Monitoring physiological responses prior to mortality, such as GC levels, may provide managers with a novel framework with which to understand neonate mortality and ecology. Managers currently use the frequency of mortality causes to reveal important details about the local ecology and conservation problems ungulates experience (Collins & Kays 2011). As a result, predation dominates ungulate neonate survival literature, primarily informing management decisions and basic ecological knowledge because predators are the primary source of ungulate neonate mortality (Linnell *et al.* 1995; Gingery *et al.* 2018). A predation and mortality focused approach, however, may result in an incomplete understanding of neonate mortality. Greater mortality and predation in the NS, for example, failed to indicate that the number or intensity of environmental stressors may have been greater in the SS (where CORT concentrations were greater) possibly because the stressors in the SS were difficult to detect or did not directly result in mortality. An individual cannot be killed directly by habitat loss, for example, making it difficult for mortality metrics to reflect responses to such stimuli. Moreover, despite evidence that predation can mask underlying factors that increase mor-

tality and mortality risk (Parker *et al.* 2009), it is rare for studies to investigate mortality outside of a detected predation event (Dion *et al.* 2020) indicating that ability of factors to influence neonate ecology and mortality susceptibility prior to death may be severely understated in the current literature. For example, in the absence of functional predators, neonatal white-tailed deer survival rates in Delaware were comparable to survival rates from other eastern populations with predators (Gingery *et al.* 2018; Dion *et al.* 2020) due to underlying diseases that were only detected after histological samples indicated disease presence. However, typically histological samples are not collected in neonate survival studies (Dion *et al.* 2020). As a result, detected mortality causes, and the frequency of those causes, may be ineffective avenues to fully assess neonate ecology and susceptibility to mortality when considered alone.

Environmental perturbations that can elicit elevated GC responses, but may not be reflected in mortality metrics, may help explain why we observed greater CORT concentrations in the SS. Caloric restriction or anthropogenic disturbances, for example, can elevate CORT concentrations (Jimeno *et al.* 2017; Zbyryt *et al.* 2017) but may not directly cause mortality or may be masked by other mortality sources. Although speculative, neonates in the SS may have been born to mothers less capable of producing enough milk during lactation and the subsequent caloric restriction may have indirectly inflated CORT concentrations. Juvenile female ungulates can struggle to reach a body mass that favors reproduction during their first breeding season (Mitchell & Brown 1974; Gerhart *et al.* 1997), leading to lower quality and quantity of milk (White 1992). In the SS, 15% more juvenile females become pregnant during their first breeding season than in the NS (Diefenbach *et al.* 2019) suggesting more neonates in the SS may have experienced a restricted food supply from young mothers because neonates are dependent on their mothers for nutrient acquisition (Verme *et al.* 1989). Furthermore, anthropogenic disturbances may elicit greater GC responses than predation-risk in ungulates (Zbyryt *et al.* 2017), which may explain why we observed greater GC concentrations in the SS. Similar to Zbyryt *et al.* (2017), CORT concentrations in the current study were greater in the SS where the potential for anthropogenic disturbances was greater due to more human activity (e.g. agriculture and human development). However, being captured in forest or agricultural areas did not explain variation in CORT concentrations (Table 1).

Additional research is needed to understand the role of CORT concentrations in neonatal ungulates. Due to limited sample size, for example, we could not estimate

parameters for a model with an interaction effect between study areas and CORT concentrations to evaluate whether CORT concentrations influenced survival differently in each study area (i.e. different intercepts and slope), although including study area was a competitive model. Given the variable range and magnitude of CORT concentrations observed in each study area, CORT concentrations may differ among populations with variable links to survival and population demographics. Research that could identify factors capable of eliciting acute and baseline CORT elevations (e.g. human disturbance, landscape variation, maternal care, caloric consumption) will be important to understanding how such factors influence both physiological and demographic responses in white-tailed deer neonates.

As studies of ungulate GC responses become more common, it will be important to investigate how such responses may influence demography (Creel *et al.* 2013; Say-Sallaz *et al.* 2019). Despite the number of studies quantifying elevated GC concentrations in adult ungulates, the effect of elevated GCs remains largely unstudied in ungulates of any age class (Creel *et al.* 2013). Biomarkers, such as CORT, can provide a better understanding of an individual's response to environmental stimuli (Edwards *et al.* 2019) and the results here suggest they may be important to understanding susceptibility to mortality. Research evaluating how GC may be linked to population demographics will provide a more comprehensive understanding of the role GCs play in ungulate populations and whether GCs may be a biomarker capable of informing population management.

ACKNOWLEDGMENTS

Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government. The authors wish to acknowledge an anonymous reviewer for their suggestion to add a model where survival was only a function of cortisol. This work was supported by the Pennsylvania Game Commission, Pennsylvania Cooperative Fish and Wildlife Research Unit, and The Pennsylvania State University.

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Cite this article as:

Gingery TM, Diefenbach DR, Pritchard CE, Ensminger DC, Wallingford BD, Rosenberry CS (2021). Survival is negatively associated with glucocorticoids in a wild ungulate neonate. *Integrative Zoology* **16**, 214–25.