File = Dip_CORT_GCE_Resubmission_ver_4.docx

Living on the edge: Glucocorticoid physiology in desert iguanas (*Dipsosaurus dorsalis*) is predicted by distance from an anthropogenic disturbance, body condition, and population density

Jessica L. Malisch *[a], Theodore Garland, Jr. [b], Laurence Claggett [a], Lindsey Stevenson [a], Ellen A. Kohl [a], Henry B. John-Alder [c]

[a] Department of Biology, St. Mary's College of Maryland, St. Mary's City, MD 20686

[b] Department of Evolution, Ecology, and Organismal Biology, University of California, Riverside, CA 92521

[c] Department of Ecology, Evolution, and Natural Resources, Rutgers University, New Brunswick, NJ 08901

*Corresponding author: E-mail: jlmalisch@smcm.edu

Keywords: body condition, conservation physiology, corticosterone, desert iguana, road ecology, stress, glucocorticoids

Abstract

Ecological factors, such as habitat quality, influence the survival and reproductive success of free-living organisms. Urbanization, including roads, alters native habitat and likely influences physiology, behavior, and ultimately Darwinian fitness. Some effects of roads are clearly negative, such as increased habitat fragmentation and mortality from vehicle collision. However, roads can also have positive effects, such as decreasing predator density and increased vegetation cover, particularly in xeric habitats due to increased water run-off. Glucocorticoids are metabolic hormones that reflect baseline metabolic needs, increase in response to acute challenges, and may mediate endogenous resource trade-offs between survival and reproduction. Here we examined circulating concentrations of corticosterone (baseline and stress-induced) in desert iguanas (*Dipsosaurus dorsalis*) in relation to the distance from a major anthropogenic disturbance, a high-traffic road in Palm Springs, CA. Additionally, we analyzed body condition and population density as additional predictors of glucocorticoid physiology. Surprisingly, we found lower baseline CORT levels closer to the road, but no effect of distance from road on stress-induced CORT or stress responsiveness (difference between baseline and stress-induced concentrations). Both population density and body condition were negative predictors of baseline CORT, stress-induced CORT, and stress responsiveness. Given the known effect of roads to increase run-off and vegetation density, increased water availability may improve available forage and shade, which may then increase the carrying capacity of the habitat and minimize metabolic challenges for this herbivorous lizard. However, it is important to recognize that surfaces covered by asphalt are not usable habitat for iguanas, likely resulting in a net habitat loss.

Introduction

Urbanization alters native habitat. An increase in development leads to an increase in traffic and the density of roads. In addition, urbanization leads to more noise pollution, light pollution, environmental toxins, and alters the temperature regime (reviewed in Andrews et al., 2008; Kociolek et al., 2011; French et al., 2018). These habitat changes alter the adaptive landscape for resident organisms and therefore influence the evolutionary trajectory of populations. For example, Western fence lizards (*Sceloporus occidentalis*) that inhabit more urban areas utilize a more diverse array of perch sites, spend more time on the ground and have modified limb and scale morphology as compared to conspecifics in natural habitats (Putman et al., 2019). These changes are related to changes in habitat structure and temperature regime and provide evidence that habitat alterations associated with urbanization can lead to changes in behavior and morphology.

Urbanization may also influence physiological traits. Because hormones respond to both external and internal stimuli and translate those stimuli into behavioral and physiological changes, they are a potential mechanistic link between an environmental challenge (“stress”) and fitness consequences (reviewed in Dantzer et al., 2014). Furthermore, glucocorticoid hormones, which increase rapidly following acute stress, are proposed to mediate the trade-off between survival and reproduction during periods of challenge (Wingfield et al., 1998). Prolonged anthropogenic stressors could influence organisms to curtail current reproduction in favor of survival, and the mean Darwinian fitness of that population could decline. However a consistent relationship between the stress response and fitness is difficult to find (see Breuner et al., 2008; Busch and Hayward, 2009; Dantzer et al., 2014 for discussion), This is likely due to the context dependency of each study, for example variation in resource availability (low vs high) may influence the relationship between the stress response and fitness (Shoenle et al., 2018; Breuner and Berk, 2019).

Roads are an anthropogenic disturbance to habitat that are increasingly prevalent and may impact organisms in numerous ways (reviewed in Andrews et al., 2008; Kociolek et al., 2011).

Roads cause habitat fragmentation and may affect animal populations by impeding movement (Marsh and Beckman, 2004; Andrews and Gibbons, 2005), increasing the risk of vehicle mortality (Andrews and Gibbons, 2005; Aresco, 2005; Kociolek et al., 2011), decreasing abundance (Fahrig and Rytwinski, 2009), decreasing genetic diversity (Clark et al., 2010), and altering the microhabitat by changing water availability (Coffin, 2007). Furthermore, vehicles traveling on roads cause noise, light, and chemical pollution (reviewed in Andrews et al., 2008; Kociolek et al., 2011). Traffic noise and other impacts of roads on ecosystems can extend beyond their immediate vicinity and are conservatively estimate to have effects 100m-800m from roads (i.e. "road-effect zone; Garland Jr. and Bradley, 1984; Forman, 2000; Forman and Deblinger, 2000; Riitters and Wickham, 2003).

Although many of the effects of roads on wildlife are negative, some alterations may benefit some species. For example, teiid lizards in the Amazon utilize anthropogenic forest clearings for basking sites and increased foraging opportunities that are limited in dense undisturbed rainforest (Sartorius et al., 1999). Snakes are known to utilize roads for basking during the day and thermoregulation at night, so commonly that road driving is utilized by professional and amateur herpetologists alike to locate specimens (e.g. Sullivan, 1981). Furthermore, roads, particularly impermeable (paved) roads, increase run off, leading to changes in community composition and plant size, which may be especially relevant for desert species that are water limited (Johnson et al., 1975; Holzapfel and Schmidt, 1990; Lightfoot and Whitford, 1991; Gelbard and Belnap, 2003). Roadside habitat has denser flower blooms (Phillips et al., 2019), an easily observable phenomenon when driving through the desert in spring. Larger vegetation stands may provide additional forage to herbivorous organisms, such as the desert iguana, and additional cover, providing refuge from the sun and aerial predators.

Several studies across vertebrate taxa have explored the relation between roads and stress physiology, particularly glucocorticoid levels. Glucocorticoids (cortisol and corticosterone; CORT) are quantified in numerous ways, including baseline (presumably not influenced by an acute stressor), stress-induced levels (CORT following some form of standardized stressor), stress reactivity (the difference or ratio of baseline and stress-induced CORT), and long-term averaged levels of CORT as measured by metabolites in fecal samples or by CORT in hair or feather samples (e.g., Wasser et al., 1997; Creel et al., 2002; Hayward et al., 2011). Interestingly, there is not a consistent model that predicts the effect of anthropogenic sources of chronic stress on directional changes in glucocorticoid parameters (reviewed in Dickens and Romero 2013; see also Schoenle et al., 2018), however, these authors emphasize that populations will respond differentially to challenges and that any disruption of the endocrine profile is indicative of chronic stress. Furthermore, these authors highlight that a decrease in body mass is the best indicator of chronic stress in wild populations (Dickens and Romero, 2013).

Most studies that have explored the relation between roads and stress physiology to date have used birds and non-reptilian species (e.g., Crino et al., 2011; Dietz et al., 2013). Owen et al., (2014) recently reported that roads are associated with numerous alterations to glucocorticoid physiology in copperhead snakes (*Agkistrodon contortrix*). Copperheads living near the road had baseline CORT levels similar to copperheads in a forested location away from a road, but the snakes near the road had lower stress-induced CORT and were thus less stress-responsive. The relationship was not explained by variation in body size. Surprisingly, baseline CORT, stress-induced CORT, and stress responsiveness were negatively associated with the number of vehicles present during the night of sample collection (Owen et al., 2014). However, additional studies in reptilian fauna are needed (French et al., 2018).

Here we investigate baseline plasma CORT concentrations, stress-induced CORT, stress-responsiveness (stress-induced minus baseline), body condition (mass/SVL) and population density in a population of desert iguanas residing adjacent to a heavily trafficked paved road in a southwestern U.S. desert. We collected samples adjacent to the road and up to 1,000 meters from the road to encompass the "road-effect-zone." Although a consistent endocrine profile for free-living organisms and chronic stress does not exist and any alteration to glucocorticoid physiology is likely an indicator of chronic stress (Dickens and Romero, 2013), we predicted that iguanas living closest to the road would have elevated baseline corticosterone levels related to visual and auditory disturbance of passing vehicles and that stress-induced CORT would also be lower, as seen in the copperhead snake study by Owen et al., 2014. Because we predict roads are a chronic stressor, we predicted proximity to roads to be related to a decrease in body condition, the most reliable indicator of chronic stress in wild animal populations according to Dickens and Romero (2013). After examining the data (see Fig. 1), we noticed that population density appeared to vary across the study site, as such we calculated the total number of neighbors in a ten meter radius for each iguana in the study and added this variable to all models. We did not have an a priori hypothesis concerning iguana density.

Methods

Study species and study location

We studied the desert iguana, Squamata: *Dipsosaurus dorsalis*, a predominantly herbivorous, medium-sized lizard (adults about 100 mm snout-vent length) that favors sandy washes in the southwestern deserts of the United States and parts of Mexico (Norris, 1953). Our study site was a 1,000m X 250m plot adjacent to Gene Autry Trail, in Palm Springs, CA (Riverside Country).

Gene Autry trail is a heavily used two-lane road, at the time of the study road traffic was approximately 20,300 vehicles per day (California Department of Transportation). The study was conducted between April and August 2004 and sampling was equally distributed between April and July, with a few samples acquired in August.

Capture and blood collection protocol

We established six parallel transects at 50 meter intervals perpendicular to the road from 0 to 1,000 meters away from the road, Gene Autry Trail (Fig. 1). Lizards were located visually while walking transects. Blood was collected from (N=159) the post-orbital sinus (Michael and Muthukkaruppan, 1984) following capture by lasso. Blood was collected into heparinized microhematocrit capillary tubes with a maximum volume of 75 μL. Blood was collected within 3 min of sighting the lizard (see Table 1). Samples were held on ice until centrifuged, and plasma was stored at −20 °C from the date of capture until assayed (~5 months). In a subset of these lizards, iguanas were held in a bag and a second blood sample was taken 15 minutes post-capture to assess adrenal responsiveness to acute stressors (N=71). Snout-vent length, sex (male, female or juvenile), body mass, and distance from road were recorded using GPS location. Lizards were marked with tail beads before released at the site of capture.

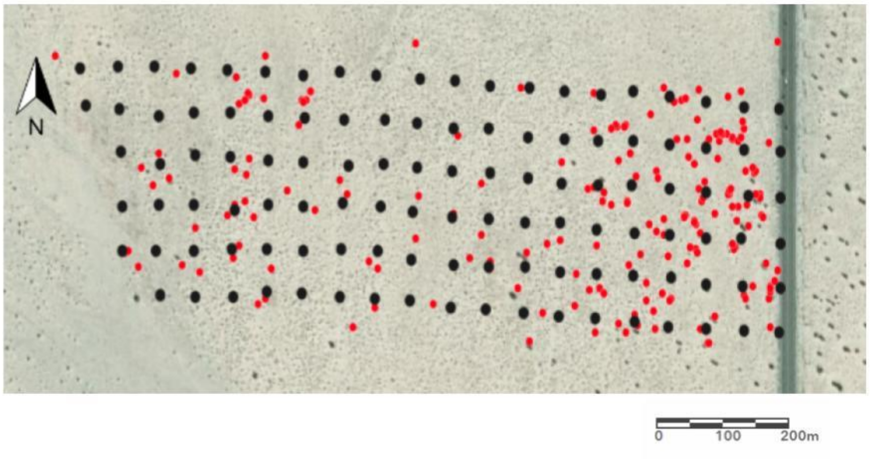

Figure 1. Study area adjacent to Gene Autry Trail, Palm Springs California. Black dots represent transect intercepts at 50 m intervals spanning 250 m of roadside and up to 1000 m from the road. Red dots represent individual lizard captures (N=159).

Plant Cover

Annual plants within a 1 meter radius of each intercept on the transect grid (black dot on Fig. 1) were measured for height, length and width and the percentage ground coverage of the sample area was calculated. A weighted percent plant cover was calculated for each iguana sampled by combining the percent plant cover at the nearest four intercepts (black dots on Fig. 1) to where the iguana was captured and adjusting for distance to each intercept.

Corticosterone Assay

Corticosterone concentration was determined by radioimmunoassay (RIAs) in the John-Alder lab at Rutgers University (see Smith and John-Alder 1999 for more detailed RIA methodology). Corticosterone from 30 ul of plasma was extracted in diethyl ether, dried, and reconstituted in phosphate buffered saline with gelatin. Reconstituted samples were assayed with 3 H-B

(PerkinElmer Life Sciences Inc., Boston, MA, USA) and B antiserum. Extraction efficiency was determined from each sample and corrected. The limit of detection was 8 pg B per assay tube. Intra-assay and inter-assay variation were 8% and 7.5%, respectively.

Statistics

All CORT values were $\log_{10}$ transformed to improve normality. The effect of acute stress (baseline sample vs 15-min post capture) on CORT was determined with a paired t-test, and the relation between baseline and stress-induced CORT was quantified with a Pearson correlation. For the effect of distance from road, we used the Mixed Procedure in SAS to implement multiple regression models while including assay batch as a random effect. Separate models were analyzed for baseline, stress-induced CORT, stress responsiveness (difference between stress-induced and baseline), and body condition. For baseline CORT, stress-induced CORT, and stress responsiveness the independent variables included sex (adult male, adult female, juvenile), distance from road, square of z-transformed distance from road (to allow for a possible non-linear relationship), body condition (mass/SVL), hematocrit, bleed delay time (time from sighting to end of blood sampling), capture date, month (as a proxy for season), capture time of day, plant cover, and population density (total number of neighbors within a 10 meter radius). For body condition, the independent variables were sex, distance from road, square of z-transformed distance from road capture date, capture month and time of day.

Results

Based on untransformed values, capture and handling increased CORT levels by >170% by 15 min post-capture (Fig. 2, Table 1; paired t-test, t_{70}=12.79, P<0.0001). Untransformed means for baseline CORT were 2.277± 1.997 ng/ml and 6.173± 4.788 ng/ml at 15 min post capture.

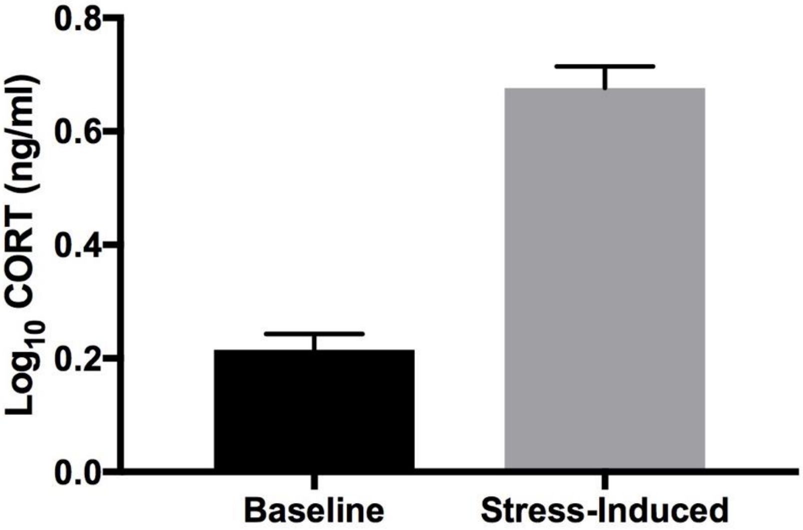

Figure 2. Mean+SEM $\log_{10}$ baseline and stress-induced (15-min post-capture) plasma CORT concentration ng/ml for 71 desert iguanas (Squamata: *Dipsosaurus dorsalis*) (paired t-test, $t_{70}=12.79$, $P<0.0001$).

Table 1. LS Means± SE of plasma corticosterone concentration for male, female and juvenile desert iguanas.

Dependent Variable	**Transform**	**Male**	**Female**	**Juvenile**
Baseline CORT (ng/ml)	Log$_{10}$	0.159±0.092	0.226±0.101	0.403±0.112
Stress-Induced CORT (ng/ml)	Log$_{10}$	0.653±0.073	0.773±0.142	0.688±0.322
Stress responsiveness (ng/ml)	Log$_{10}$	0.413±0.061	0.577±0.219	0.496±0.354

Table 2. Simple means, SEM, N, minimum and maximum values for covariates.

Variable	**Mean**	**SEM**	**N**	**Min**	**Max**
Distance from road (m)	292	264	159	1.0	981
Body mass (g)	46.33	4.74	159	7.0	82.0
SVL (mm)	111.2	13.6	157	66.0	136.0
Bleed delay time (min)	2.03	0.86	159	0.87	6.17
Baseline HCT (%RBC)	32.7	6.5	159	17.7	69.1
Stress-induced HCT (%RBC)	33.1	6.7	71	16.7	69.2
Weighted plant cover (%)	23.2	15.3	159	0.0	86.4
Number of neighbors within 10 m	0.64	1.06	159	0.0	5

Baseline CORT (ng/ml) and stress-induced CORT were positively correlated: lizards with higher baseline CORT had higher stress-induced CORT (Fig. 3, r= 0.5163, P<0.0001).

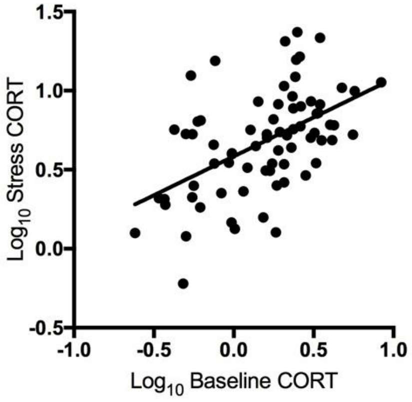

Figure 3. Positive relation between log transformed baseline CORT (ng/ml) and log transformed stress-induced CORT (ng/ml) in desert iguanas (R^2= 0.2666, P<0.0001, n=73).

Adjusting for the various covariates, baseline CORT (ng/ml) differed among the three sex categories (ANCOVA, $F_{2,131}$=5.20, P_{sex}=0.0067, Fig. 4, Tables 1,3). Distance from road was a positive predictor of baseline CORT (ANCOVA, $F_{1,131}$=5.94, $P_{distance}$=0.0016, Fig. 5, Table 3). Body condition (Mass/SVL) was a negative predictor of baseline CORT (ANCOVA, $F_{1,131}$=6.75, $P_{body\ condition}$=0.0104, Table 3): lizards in better body condition had lower baseline CORT. Lizard density was also a negative predictor of baseline CORT (ANCOVA, $F_{1,131}$=4.03, $P_{density}$=0.0468,Table 3.) Baseline HCT, bleed delay time, date, month, and plant cover did not predict baseline CORT (see Table 3). Models were repeated without non-significant variables and the results did not change.

Table 3. Analysis of covariance for log-transformed baseline plasma corticosterone levels in desert iguanas (Squamata: *Dipsosaurus dorsalis*). Assay batch (N=6) was included as a random effect. N=149.

Effect	**Estimate**	**S.E.**	**df**	**F**	**P**
Intercept	-374.93	343.05			
Sex (F, M, Juvenile)			2,131	5.20	**0.0067**
Sex=1=Male	-0.0676	0.05959			
Sex=2=Juvenile	0.1767	0.08464			
Distance from Road (m)	0.0004	0.00016	1,131	5.94	**0.0162**
Squared Distance from Road (m)	-0.0531	0.03121	1,131	2.90	0.0912
Baseline HCT (%RBC)	0.6754	0.39700	1,131	2.89	0.0912
Bleed delay time (min)	-0.0138	0.00032	1,131	0.24	0.6251
Time of Day (min)	-0.0005	0.02824	1,131	2.66	0.1054
Date	0.0032	0.00292	1,131	1.21	0.2736
Month	-0.1213	0.08589	1,131	1.99	0.1602
Weighted Plant cover (%)	0.0031	0.00170	1,131	3.26	0.0734
Condition (Mass/SVL) (g/mm)	-53.416	20.5547	1,131	6.75	**0.0104**
Number of neighbors within 10 m	-0.0483	0.02408	1,131	4.03	**0.0468**

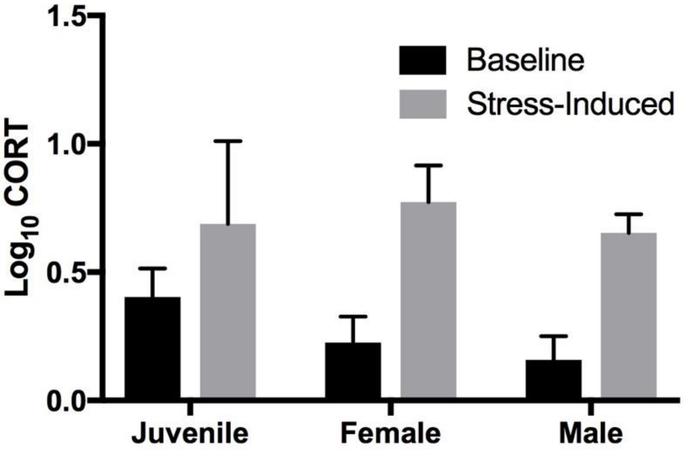

Figure 4. LS means ± SEM of $\log_{10}$ Baseline CORT (ng/ml) and log stress-induced CORT (ng/ml) in juvenile, female, and male desert iguanas (Squamata: *Dipsosaurus dorsalis*). The values are from the ANCOVA models shown in Tables 3 and 4. The groups differ in baseline (Table 3, P_{sex}=0.0067) but not in stress-induced CORT (Table 4, P_{sex}=0.6920).

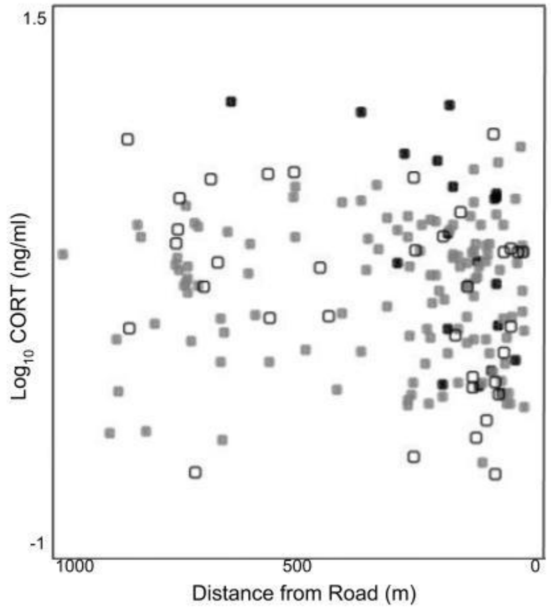

Figure 5. Log_{10} baseline CORT (ng/ml) and distance from road for females (open squares, sex=0), males (grey squares, sex=1) and juveniles (black squares, sex=2) (N=159).

Stress-induced CORT (ng/ml) did not differ among the three sex categories (ANCOVA, $F_{2,51}$=0.27, P_{sex}=0.6920, Fig. 4., Table 1,4); however, the sample size for stress-induced CORT for both females and juveniles was low (N = 7 and 2, respectively). Distance from road was not a predictor of stress-induced CORT (ANCOVA, $F_{1,51}$=0.14, $P_{distance}$=0.7095, see Table 4). Body condition tended to be a negative predictor of stress-induced plasma CORT concentration (ANCOVA, $F_{1,51}$=3.74, $P_{condition}$=0.0587, Table 4), iguanas in lower body condition tended to have higher stress-induced plasma CORT. Lizard density was a negative predictor of stress-induced CORT (ANCOVA, $F_{1,51}$=6.24, $P_{density}$=0.0157, Table 4). HCT, bleed delay time, date, month, and plant cover did not predict stress-induced CORT (see Table 4). Models were repeated without non-significant variables and the results did not change.

Table 4. Analysis of covariance for log-transformed stress-induced plasma corticosterone levels in desert iguanas (Squamata: *Dipsosaurus dorsalis*). Assay batch (N=6) was included as a random effect. N = 69.

Effect	**Estimate**	**S.E.**	**df**	**F**	**P**
Intercept	203.82	492.51			
Sex (F, M, Juvenile)			2,51	0.37	0.6920
Sex=1=Male	-0.1199	0.1400			
Sex=2=Juvenile	-0.0847	0.3439			
Distance from Road (m)	0.0001	0.0003	1,51	0.14	0.7095
Squared Distance from Road (m)	-0.0465	0.0466	1,51	1.00	0.3299
Stress-induced HCT (%RBC)	0.2278	0.5911	1,51	0.15	0.7015
Bleed delay time (min)	0.0243	0.0456	1,51	0.28	0.5967
Time of Day (min)	0.0004	0.0006	1,51	0.43	0.5172
Date	-0.0017	0.0042	1,51	0.17	0.6844
Month	0.0876	0.1315	1,51	0.44	0.5081
Weighted Plant cover (%)	-0.0037	0.0028	1,51	1.78	0.1880
Condition (Mass/SVL) (g/mm)	-71.538	36.994	1,51	3.74	0.0587
Number of neighbors within 10 m	-0.1049	0.0420	1,51	6.24	**0.0157**

Stress responsiveness did not differ among the three sex categories (ANCOVA, $F_{2,50}$=0.26, P_{sex}=0.7700, Table 1,5). Distance from road was not a predictor of stress responsiveness (ANCOVA, $F_{1,50}$=0.12, $P_{distance}$=0.7306, Table 5). Both body condition (ANCOVA, $F_{1,50}$=4.85, $P_{condition}$=0.0323) and lizard density (ANCOVA, $F_{1,50}$=5.42, $P_{density}$=0.0240) were negative predictor of stress responsiveness (Table 5). HCT, bleed delay time, date, month, and plant cover did not predict stress responsiveness (see Table 5). Models were repeated without non-significant variables and the results did not change.

Table 5. Analysis of covariance for stress-responsiveness (stress-induced minus baseline) of plasma corticosterone levels in desert iguanas (Squamata: *Dipsosaurus dorsalis*). Assay batch (N=6) was included as a random effect. N=68.

Effect	**Estimate**	**S.E.**	**df**	**F**	**P**
Intercept	384.54	800.54			
Sex (F, M, Juvenile)			2,50	0.26	0.7700
Sex=1=Male	-0.1646	0.2329			
Sex=2=Juvenile	-0.0817	0.4107			
Distance from Road (m)	-0.0002	0.0004	1,50	0.12	0.7306
Squared Distance from Road (m)	-0.0171	0.0768	1,50	0.05	0.8242
Stress-induced HCT (%RBC)	-1.3629	1.4928	1,50	0.83	0.3839
Bleed delay time (min)	0.1082	0.0717	1,50	2.28	0.1377
Time of Day (min)	0.0012	0.0009	1,50	1.58	0.2151
Date	-0.0033	0.0068	1,50	0.23	0.6358
Month	0.2410	0.2064	1,50	1.36	0.2484
Weighted Plant cover (%)	-0.0085	0.0046	1,50	3.36	0.0729
Condition (Mass/SVL) (g/mm)	-122.29	55.540	1,50	4.85	**0.0323**
Number of neighbors within 10 m	-0.1556	0.0668	1,50	5.42	**0.0240**

Body size (SVL) differed by sex (ANCOVA, $F_{2,146}$=88.16, $P_{sex} << 0.0001$ (LS mean males = 117.0±0.89 mm, females = 109.4±1.54 mm, juveniles = 86.6±2.07 mm) but not in relation to distance from road, date, plant cover or number of nearest neighbors. Body condition did not differ by sex (ANCOVA, $F_{2,146}$=2.66, P_{sex}=0.0731 (LS mean males = 0.03195±0.0001 g/mm, females = 0.03175±0.0002 g/mm, juveniles = 0.03128±0.0002 g/mm) nor any covariates.

Discussion

Urbanization modifies habitat and can therefore alter the adaptive landscape for resident organisms. Here we examined potential changes in physiological traits, specifically, baseline, stress-induced, and stress-responsiveness of glucocorticoids in desert iguanas living at varying distances from a high traffic road. We expected to see a detrimental effect of roads on desert iguanas, but our results were not consistent with our predictions. We found lower baseline CORT in lizards living in closer proximity to the road but no effect of road proximity on stress-induced

CORT or stress-responsiveness. Furthermore, we found that baseline CORT, stress-induced CORT and stress responsiveness were all negatively associated with population density. Finally, body condition was negatively related to baseline CORT and stress responsiveness and tended to also be associated with lower stress-induced CORT. However, it should be noted that this study focused on a single study site with an associated road. Additional replication and experimental approaches are needed to draw robust general conclusions and to disentangle potentially correlated factors.

Interestingly, baseline CORT was positively associated with distance from road (Fig. 4), lizards residing closer to the road also had low baseline CORT. There are several possible explanations: e.g., lower baseline CORT may be an adaptation (evolutionary response) to living adjacent to disturbance. Another possibility is that iguanas are genetically predisposed to low baseline CORT and therefore may be more resilient to the challenges of anthropogenic disturbance and thus are better able to inhabit disturbed areas. Additionally, this habitat may provide better resources and therefore lower baseline CORT reflects a lower energetic challenge. Additionally, low baseline CORT may reflect a deleterious response to chronic stress (reviewed in Dickinson and Romero, 2013). These hypotheses are not mutually exclusive and cannot be fully addressed by the current study. However, when all major predictors of glucocorticoid physiology from this study are examined collectively, notably the association of low baseline CORT with good body condition as well as higher population density, the most parsimonious explanation suggests to us, that the habitat in this study closest to the road provides some form of energetic benefit to desert iguanas. However, this study examined one population along one stretch or road, therefore these results require replication.

These apparent benefits of living near the road may be related to plant type, density or size. Desert iguanas are frequently associated with creosote shrubs which were higher in abundance near the road as well as at points more distant from the road (pers. obs.). Increased run off is associated with larger vegetation stands, particularly in desert habitats (Johnson et al., 1975; Lightfoot and Whitford, 1991). Johnson et al. (1975) estimated that desert productivity is 17 times higher in desert plants adjacent to a paved road. This is particularly beneficial to herbivorous organisms like desert iguanas. Indeed, it was noticeably easier to locate iguanas in transects within 100 m from the road edge (pers. obs.): despite increased sampling efforts at areas more distant from the road, 28% of all lizards in this study were found within 100 meters of the road and 67% were within 316 meters of the road edge. Creosote bush (*Larrea tridentata*), which is dominant at this study site, is known to be larger and host a larger population of herbivores insects in bushes adjacent to the road as compared with bushes 20 m out from the road (Lightfoot and Whitford, 1991). In this study we quantified plant cover and utilized it as a covariate, but it did not predict any CORT metrics (see Tables 3,4 and 5). However, because the vegetation in deserts is low and we quantified plant cover for 1 square meter around each transect point, we suspect that our plant cover metric was not of sufficient scale to detect the variation in plant cover that was obvious by visual inspection alone.

Desert iguanas were also in higher abundance near the road than at points more distant from the road with sparse sighting and captures in the middle third of the study plot (see Fig. 1). Baseline CORT was negatively associated with population density, a surprise because high population density is typically a stressor (e.g., Haenel et al., 2003). However, if resources are higher by the road (i.e., more water from runoff, larger and more dense annual plants) then habitat quality may influence iguana distribution. It is possible that iguana populations are higher in high quality areas and that is reflected in lower baseline CORT. Another possibility is that iguanas inhabiting locations further from the road have larger home range size, which could be associated with higher baseline CORT. Both of these possibilities should be investigated in future studies.

Body condition was a negative predictor of baseline CORT and stress responsiveness, and tended to also negatively predict stress-induced CORT (see Tables 3,4,and 5). Iguanas in better condition as measured by Mass/SVL, had lower CORT. This finding is consistent with several other studies that have reported a negative relation between body condition and baseline CORT. For example, body condition is a negative predictor of baseline CORT in male Red-spotted garter snakes (Moore et al., 2000), free baseline CORT in tufted puffins (Williams et al. 2008), female eiders (Jaatinen et al., 2013) brown tree snakes (Waye and Mason, 2008), and the common lizard, *Lacerta vivipara* (Oppliger et al. 1998). In marine iguanas, baseline CORT was elevated in years of low forage availability and high CORT 15 min post capture was also a negative predictor of survival (Romero and Wikelski, 2001). Therefore, our findings likely indicate that iguanas in better condition have access to better resources which is reflected in lower baseline CORT.

Other than population density, none of our predictor variables were predictors of stress-induced CORT. This is not consistent with Owens et al. (2014) who reported higher stress-induced CORT and therefore also increased stress responsiveness (stress-induced CORT – baseline CORT) in copperheads found on roads as compared to snakes located away from roads in the woods. We did see a trend for stress-induced CORT to be higher in individuals with lower body condition (see Table 4), suggesting that iguanas with access to fewer resources mobilize more glucocorticoid hormones in response to acute challenge.

In conclusion, an anthropogenic disturbance, a high-traffic road, is associated with altered glucocorticoid physiology in the desert iguana. The main finding here is iguanas living in closer proximity to the road have lower baseline CORT. Furthermore, lower baseline CORT is associated with better body condition and higher population density, but no effect on body size. Given the lack of effect on body size, we assume that the road is not acting as a population sink due to mortality. Taken together, these results suggest that a road has the potential to alter the adaptive landscape for this species and has the potential to influence the evolution of physiological traits in desert iguanas.

Acknowledgments

We would like to thank three anonymous reviewers whose comments improved the manuscript. We thank W. Hodges for assistance in the field and help with experimental design. We also thank Robert Cox for lab assistance during hormone analyses. Funding for this project was provided through a Shipley-Skinner Grant awarded to JLM through The Center for Conservation Biology at UCR. This work was approved by UCR-IACUC and covered by a CDFG scientific collection permit to JLM.

References

Andrews, K.M., Gibbons, J.W., 2005. How do highways influence snake movement? Behavioral responses to roads and vehicles. Copeia 4, 772-782.

Andrews, K.M., Gibbons, J.W., Jochimsen, D.M., 2008. Ecological effects of roads on amphibians and reptiles: a literature review. Herpetol. Conserv. 3, 121-143.

Aresco, M.J., 2005. Mitigation measures to reduce highway mortality of turtles and other herpetofauna at a north Florida lake. J. Wildl. Manage. 69, 549-560.

Bailey, F.C., Cobb, V.A., Rainwater, T.R., Worrall, T., Klukowski, M., 2009. Adrenocortical Effects of Human Encounters on Free-Ranging Cottonmouths (*Agkistrodon piscivorus*). J. Herpetol. 43, 260-266.

Breuner, C.W., Berk, S.A., 2019. Using the van Noordwiik and de Jong resource framework to evaluate glucocorticoid-fitness hypotheses. Intg. Comp. Biol.

Breuner, C.W., Patterson, S.H., Hahn, T.P., 2008. In search of relationships between the acute adrenocortical response and fitness. Gen. Comp. Endocrinol. 157, 288-295.

Busch, D.S., Hayward, L.S., 2009. Stress in a conservation context: a discussion of glucocorticoid actions and how levels change with conservation-relevant variables. Biol. Conserv. 142, 2844-2853.

Clark, R.W., Brown, W.S., Stechert, R., Zamudio, K.R., 2010. Roads, interrupted dispersal, and genetic diversity in timber rattlesnakes. Conserv. Biol. 24, 1059-1069.

Coffin, A.W., 2007. From roadkill to road ecology: a review of the ecological effects of roads. J Transport Geography 15, 396-406.

Cooke, S.J., Sack, L., Franklin, C.E., Farrell, A.P., Beardall, J., Wikelski, M., Chown, S.L., 2013. What is conservation physiology? Perspectives on an increasingly integrated and essential science. Conserv. Physiol. 1, cot001.

Creel, S., Fox, J.E., Hardy, A.J., Sands, J., Garrott, B., Peterson, and R.O., 2002. Snowmobile activity and glucocorticoid stress responses in wolves and elk. Conserv. Biol. 16, 809-814.

Crino, O., Van Oorschot, B.K., Johnson, E., Malisch, J.L., Breuner, C.W., 2011. Proximity to a high traffic road: glucocorticoid and life history consequences for nestling white-crowned sparrows. Gen. Comp. Endocrinol. 173, 323-332.

Dantzer, B., Fletcher, Q.E., Boonstra, R., Sheriff, M.J., 2014. Measures of physiological stress: a transparent or opaque window into the status, management and conservation of species? Conserv. Physiol. 2, cou023.

DeNardo, D.F., Sinervo, B., 1994a. Effects of corticosterone on activity and home-range size of free-ranging male lizards. Horm. Behav. 28, 53-65.

DeNardo, D.F., Sinervo, and B., 1994b. Effects of steroid hormone interaction on activity and home-range size of male lizards. Horm. Behav. 28, 273-287.

Dickens, M. J., Romero, L.M., 2013. A consensus endocrine profile for chronically stressed wild animals does not exist. Gen. Comp. Endocrinol. 191, 177-189.

Dietz, M.S., Murdock, C.C., Romero, L.M., Ozgul, A. , Foufopoulos, J., 2013. Distance to a road is associated with reproductive success and physiological stress response in a migratory landbird. Wilson J. Ornithol. 125, 50-61.

Dunlap, K.D., Wingfield, J.C., 1995. External and internal influences on indices of physiological stress. I. Seasonal and population variation in adrenocortical secretion of free-living lizards, *Sceloporus occidentalis*. J. Exp. Zool. 271, 36-46.

Fahrig, L., Rytwinski, T., 2009. Effects of roads on animal abundance: an empirical review and synthesis. Ecol. Soc. 14 (1).

Forman, R.T., 2000. Estimate of the area affected ecologically by the road system in the United States. Conserv. Biol. 14, 31-35.

Forman, R.T., Deblinger, R.D., 2000. The ecological road-effect zone of a Massachusetts (USA) suburban highway. Conserv. Biol. 14, 36-46.

French, S.S., Webb, A.C., Hudson, S.B., Virgin, E.E., 2018. Town and country reptiles: A review of reptilian responses to urbanization. Integ. Comp. Biol. 58, 948-966.

Garland Jr, T., Bradley, W.G., 1984. Effects of a highway on Mojave Desert rodent populations. Am. Midl. Nat. 1984, 47-56.

Gelbard, J.L., Belnap, J., 2003. Roads as conduits for exotic plant invasions in a semiarid landscape. Conserv. Biol. 17, 420-432.

Haenel, G.J., Smith, L.C., John-Alder, H.B., 2003. Home-range analysis in Sceloporus undulatus (Eastern Fence Lizard). I. Spacing Patterns and the context of territorial behavior. Copeia 2003, 99-112.

Hayward, L.S., Bowles, A.E., Ha, J.C., Wasser, S.K., 2011. Impacts of acute and long-term vehicle exposure on physiology and reproductive success of the northern spotted owl. Ecosphere 2, 1-20.

Holzapfel, C., Schmidt, W., 1990. Roadside vegetation along transects in the Judean desert. Israel J. Botany 39, 263-270.

Huyghe, K., Husak, J.F., Herrel, A., Tadic, Z., Moore, I.T., Van Damme, R., Vanhooydonck, B., 2009. Relationships between hormones, physiological performance and immunocompetence in a color-polymorphic lizard species, *Podarcis melisellensis*. Horm. Behav. 55, 488-494.

Jaatinen, K., Seltmann, M.W., Hollmén, T., Atkinson, S., Mashburn, K., Öst, M., 2013. Context dependency of baseline glucocorticoids as indicators of individual quality in a capital breeder. Gen. Comp. Endocrinol. 191, 231-238.

Johnson, H.B., Vasek, F.C., Yonkers, T., 1975. Productivity, diversity and stability relationships in Mojave Desert roadside vegetation. Bulletin of the Torrey Botanical Club, 106-115.

Kociolek, A., Clevenger, A., St Clair, C., Proppe, D., 2011. Effects of road networks on bird populations. Conserv. Biol. 25, 241-249.

Lightfoot, D.C., Whitford, W.G., 1991. Productivity of creosotebush foliage and associated canopy arthropods along a desert roadside. Am. Midl. Nat., 310-322.

Madliger, C.L., Love, O.P., 2015. The Power of Physiology in Changing Landscapes: Considerations for the Continued Integration of Conservation and Physiology. Integ. Compar. Biol. 55, 545-553.

Malisch, J.L., Bennett, D.J., Davidson, B.A., Wenker, E.E., Suzich, R.N., Johnson, E.E., 2018. Stress-Induced Hyperglycemia in White-Throated and White-Crowned Sparrows: A New Technique for Rapid Glucose Measurement in the Field. Physiol. Biochem. Zool. 91, 943-949.

Marsh, D.M., Beckman, N.G., 2004. Effects of forest roads on the abundance and activity of terrestrial salamanders. Ecol. Appl. 14, 1882-1891.

Michael, R.D., Muthukkaruppan, V.R., 1984. A simple method for serial bleeding of lizards. Develop. Comp. Immunol. 8, 209-212.

Moore, I.T., Lerner, J.P, Lerner, D.T., Mason, R.T., 2000. Relationships between annual cycles of testostoerone, corticosterone, and body condition in make red-spotted garter snales, Thamnophis sirtalis concinnus. Physiol. Biochem. Zool. 73, 307-312.

Oppliger, A., Clobert, J., Lecomte, J., Lorenzon, P., Boudjemadi, K., John-Alder, H.B., 1998. Environmental stress increases the prevalence and intensity of blood parasite infection in the common lizard *Lacerta vivipara*. Ecol. Lett. 1, 129-138.

Owen, D.A., Carter, E.T., Holding, M.L., Islam, K., Moore, I.T., 2014. Roads are associated with a blunted stress response in a North American pit viper. Gen. Comp. Endocrinol. 202, 87-92.

Polich, R.L. 2016. Stress hormone levels in a freshwater turtle from sites differing in human activity. Conserv. Physiol. 4, cow016.

Phillips, B.B., Gaston, K.J., Bullock, J.M., Osborne, J.L., 2019. Road verges support pollinators in agricultural landscapes, but are diminished by heavy traffic and summer cutting. J. Applied Ecol., *56*, 2316-2327.

Putman, B.J., Gasca, M., Blumstein, D.T., Pauly, G.B., 2019. Downsizing for downtown: limb lenghts, toe lengths, and sclare counts decrease with urbanization in Western fence lizards (*Sceloporus occidentalis*). Urban Ecosystems, 1-11.

Riitters, K.H., Wickham, J.D., 2003. How far to the nearest road? Front. Ecol. Environ. 1, 125-129.

Sartorius, S.S., Vitt, L.J., Colli, G.R., 1999. Use of naturally and anthropogenically disturbed habitats in Amazonian rainforest by the teiid lizard *Ameiva ameiva*. Biol. Conserv. 90, 91-101.
Singleton, J.M., Garland Jr., T., 2018. Among-individual variation in Desert Iguanas (Squamata: *Dipsosaurus dorsalis*): endurance capacity is positively related to home range size. Physiol. Biochem. Zool. 91, 725-730.

Schoenle, L.A., Zimmer, C., Vitousek, M.N., 2018. Understanding context dependance in glucocorticoid-fitness relationships: the role of the nature of the challenge, the intensity and frequency of stressors, and life history. Integ. Comp. Biol. 58, 777-789.

Sullivan, B.K., 1981. Distribution and relative abundance of snakes along a transect in California. J. Herpetol. 15, 247-248.

Wasser, S.K., Bevis, K., King, G., Hanson, E., 1997. Noninvasive physiological measures of disturbance in the northern spotted owl. Conserv. Biol. 11, 1019-1022.

Waye, H.L., Mason, R.T., 2008. A combination of body condition measurements is more informative than conventional condition indices: temporal variation in body condition and corticosterone in brown tree snakes (*Boiga irregularis*). Gen. Comp. Endocrinol. 155, 607-612.

Williams, C.T., Kitaysky, A.S., Kettle, A.B., Buck, C.L., 2008. Corticosterone levels of tufted puffins vary with breeding stage, body condition index, and reproductive performance. Gen. Comp. Endocrinol. 158, 29-35.

Wilson, B.S., Wingfield, J.C., 1994. Seasonal and Interpopulational Variation in Plasma-Levels of Corticosterone in the Side-Blotched Lizard (*Uta Stansburiana*). Physiol. Zool. 67, 1025-1049.

Wingfield, J.C., Maney, D.L., Breuner, C.W., Jacobs, J.D., Lynn, S., Ramenofsky, M., Richardson, R.D., 1998. Ecological bases of hormone—behavior interactions: the "emergency life history stage". American Zool. 38, 191-206.

Wingfield, J.C., Vleck, C.M., Moore, M.C., 1992. Seasonal changes of the adrenocortical response to stress in birds of the Sonoran Desert. J. Exp. Zool. A: Ecol. Genet. Physiol. 264, 419-428.

Zena, L.A., Dillon, D., Hunt, K.E., Navas, C.A., Bícego, K.C., Buck, C.L., 2018. Seasonal changes in plasma concentrations of the thyroid, glucocorticoid and reproductive hormones in the tegu lizard *Salvator merianae*. Gen. Comp. Endocrinol. 273, 134-143.