General and Comparative Endocrinology 202 (2014) 87–92

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Roads are associated with a blunted stress response in a North American pit viper

Dustin A.S. Owen [a,*], Evin T. Carter [b], Matthew L. Holding [c], Kamal Islam [a], Ignacio T. Moore [d]

[a] Department of Biology, Ball State University, Muncie, IN 47306, USA
[b] Department of Ecology & Evolutionary Biology, University of Tennessee-Knoxville, Knoxville, TN 37996, USA
[c] Department of Evolution, Ecology, and Organismal Biology, The Ohio State University, Columbus, OH 43210, USA
[d] Department of Biological Sciences, Virginia Tech, Blacksburg, VA 24061, USA

ARTICLE INFO

Article history:
Received 19 October 2013
Revised 23 March 2014
Accepted 10 April 2014
Available online 4 May 2014

Keywords:
Agkistrodon contortrix
Conservation
Corticosterone
Ecophysiology
Stress response
Traffic

ABSTRACT

Whereas numerous studies have examined roads as anthropogenic stressors in birds and mammals, comparatively few studies have been undertaken on reptiles. We investigated plasma corticosterone (CORT) levels at baseline and following 30 min of restraint stress in free-ranging copperhead snakes (*Agkistrodon contortrix*) captured within the forest interior or while in contact with public roads. There was no difference in baseline CORT levels between snakes in the forest and on roads. Copperheads responded to restraint stress by increasing plasma levels of CORT; however snakes on roads exhibited a lower CORT stress response compared to forest snakes. Additionally, among snakes captured on roads there was a negative association between road traffic and baseline CORT, stressed CORT, and the magnitude of the CORT response. Our results suggest that roads are associated with a blunted stress response in copperheads. Reduced stress responses may be indicative of acclimation, the inhibited ability to mount a stress response in the face of prolonged chronic stress, or that road environments select for individuals with lower CORT responsiveness. Either scenario could result in increased road mortality if snakes do not perceive roads as a potential threat.

1. Introduction

Roads are a highly influential form of human disturbance at local and landscape scales. The incursion of roads into natural areas has been linked to population declines in many vertebrate systems through direct habitat loss associated with roads, encroachment of 'edge' species into habitat fragments, and mortality from motor vehicles (Marsh and Beckman, 2004; Kociolek et al., 2011). The mortality of terrestrial vertebrates due to road traffic has been documented extensively (review in Fahrig and Rytwinski, 2009). However, we remain relatively uninformed about how individual organisms respond behaviorally and physiologically to roads. Knowledge of individual stress physiology could reveal a proximate cause of population-level trends and yield valuable insight for the design and implementation of road-crossing structures for wildlife.

Glucocorticoid stress hormones, such as corticosterone (CORT), can provide both current and long-term information on the physiological responses of individuals and populations to environmental perturbations (Romero, 2004; Busch and Hayward, 2009; Sheriff et al., 2011). At baseline levels, CORT's primary role is thought to be metabolic, regulating energy reserves in response to current and anticipated energy demands (Moore and Jessop, 2003; Romero, 2004). Acute or extended periods of stress results in a stress response, characterized by increased levels of plasma CORT that are thought to promote survival until the stress passes (Bonier et al., 2009). However, chronic stress can lead to a depletion of stored energy resulting in allostatic overload (negative energy balance, sensu McEwen and Wingfield, 2003; Goymann and Wingfield, 2004). Prolonged allostatic overload (chronic stress) can have direct, negative fitness consequences (Wingfield and Romero, 2001; Romero, 2004; Bonier et al., 2009). With knowledge of individual plasma CORT levels, inferences can be made on a populations' current condition, facilitating conservation practices (Moore et al., 2005; Busch and Hayward, 2009). Because landscape alterations are known causes of increased stress in some organisms (Crino et al., 2011; Brearley et al., 2012; Lucas and French, 2012), glucocorticoids can be used to assess the sub-lethal effects of these alterations on natural populations.

There is some precedence for evaluating the impacts of roads from the perspective of stress physiology. Elevated hair sample

* Corresponding author. Address: Center of Excellence for Field Biology, Department of Biology, Austin Peay State University, P.O. Box 4718, Clarksville, TN 37044, USA.

E-mail address: dasowen27@gmail.com (D.A.S. Owen).

http://dx.doi.org/10.1016/j.ygcen.2014.04.020
0016-6480/

cortisol levels were documented in squirrel gliders (*Petaurus norfolcensis*) from populations adjacent to major roads (Brearley et al., 2012). Heightened stress reactivity was also found in male mountain white-crowned sparrows near roads (*Zonotrichia leucophrys oriantha*) (Dietz et al., 2013). Off-highway vehicle exposure and close proximity to logging roads are associated with increased fecal glucocorticoid levels in northern spotted owls (*Strix occidentalis caurina*) (Wasser et al., 1997; Hayward et al., 2011) Further, increased traffic levels are positively associated with elevated fecal cortisol levels in elk (*Cervus elaphus*) and wolves (*Canis lupus*) (Creel et al., 2002), pointing to a direct role of automobile traffic as a potential stressor rather than the road itself. Such studies imply that proximity to roads and motor vehicles are associated with increased baseline and stress-induced levels of glucocorticoids in a wide variety of taxa. However, no studies have addressed the impact(s) of roads and motor vehicles on a reptile, and yet reptiles are often encountered on roads with many herpetologists using roads to search for them.

Reptiles represent a taxonomic group of particular interest with regard to road interactions, as they are ground-dwelling, often slow-moving, and suffer serious road-associated mortality that can contribute to the plight of threatened species (Roe et al., 2006). Road mortality claims large numbers of snakes and turtles (Roe et al., 2006), and has been associated with reduced gene flow in some taxa (e.g., snakes: Clark et al., 2010). It is believed that road surfaces attract some species of reptiles for thermoregulatory purposes (Andrews et al., 2008), exposing them to the risk of vehicular mortality. Roads have also been documented as behavioral barriers, inhibiting movement/dispersal, in many species of snakes (Shine et al., 2004; Andrews and Gibbons, 2005; Shepard et al., 2008) and some turtles (Shepard et al., 2008). It is plausible that avoidance of roads is a result of the significantly decreased ground cover associated with roads, and the resulting perceived increase in predation risk associated with crossing such an open space (Shine et al., 2004).

Copperheads (*Agkistrodon contortrix*) represent a useful organism for assessing the impacts of roads, as they are locally abundant across much of the eastern temperate forest region of North America and are known to exhibit a high frequency of road crossing throughout the Midwest (Minton, 2001; Carter, 2012). They are not territorial, typically breed during late August – late October (Minton, 2001), and their mean home range size within our study area has been reported to range between 4 and 16 ha based on radiotelemetry data (Carter, 2012). Additionally, their habit of lying motionless when encountered makes them more susceptible to vehicular mortality, but also facilitates capture. If close proximity to roads or the associated traffic are stressors, this should manifest in altered baseline CORT and/or CORT stress response. Based on similar studies (Rich and Romero, 2005; Cyr and Romero, 2007), we tested the predictions that: (1) baseline CORT levels will be higher, and CORT stress responses will be lower, in copperheads captured on roads compared to copperheads captured in the forest (indicative of chronic stress), and (2) higher amounts of traffic will result in increased baseline and decreased CORT stress responses for copperheads captured on roads.

2. Materials and methods

2.1. Study sites

This study took place at three primary locations in southern Indiana: Clark State Forest (38°32′51″ N, 085°56′00″ W; elevation ~271 m), Morgan–Monroe State Forest (39°22′45″ N, 086°25′30″ W; elevation ~263 m), and Yellowwood State Forest (39°12′18″ N, 086°20′45″ W; elevation ~193 m). Each site consists of approximately 9700 ha within the Knobstone Escarpment section of the Norman Upland, a region characterized by steep ridges and valleys dominated by mature oak-hickory forest (www.in.gov/dnr). These sites are representative of the primary habitat utilized by copperheads throughout Indiana (Minton, 2001). Morgan–Monroe State Forest is situated just north of Yellowwood State Forest, and the two sites are only partially divided from one another by a 3 km wide mosaic of public and private land. Clark State Forest lies to the southeast of Yellowwood State Forest with approximately 80 km of private and public land separating them. As Indiana State Forests, each site is open to the public 24-h per day and receives continuous traffic via both state and county roadways. Roads used during this study consisted of mostly paved stretches of road, with some portions transitioning into gravel.

2.2. Sample collection

Copperheads ($n = 29$) were captured from Clark ($n = 16$), Morgan–Monroe ($n = 6$), and Yellowwood ($n = 7$) state forest from 15-May to 8-Sept-2012. Study sites were randomly selected each week, with specific roads (road snakes) and locations (forest snakes) of preferred copperhead habitat being randomly surveyed. We located snakes on roads ($n = 20$) by driving on them at night, using the automobile headlights to search for snakes. Visual searching on foot was employed to locate snakes in the forest ($n = 9$) using LED headlamps within areas where snakes were known to occur (Carter, 2012). Snakes were captured on both paved ($n = 18$) and gravel ($n = 2$) roads. There was no significant difference between gravel and paved snakes, and removal of gravel road snakes did not influence our results, so we included them in our analyses. We captured snakes on roads only if at least half of their body was in contact with the road's surface, while snakes captured in the forest were included only if they were at least 100 m away from a road to minimize the potential influences of roads and vehicles (Dietz et al., 2013). While Dietz et al. (2013) did not detect a significant difference in the stress levels of a migratory land-bird greater than 20 m from roads; we selected 100 m to have a more conservative cut-off. All searching was conducted at night, between the hours of 2100 and 0200, to minimize potential diel variations in CORT (Jones and Bell, 2004). During each capture, we recorded sex (via cloacal probing), female reproductive condition (via palpation for embryos), snout-vent length (SVL; ±1 cm), mass (±2 g) via spring scale (Pesola-Scales.com, Kapuskasing, Ontario, Canada), air and ground surface temperature (°C) via Kestrel Wind Meter (Loftopia LLC, Birmingham MI, USA), date, and time. We also calculated a body condition index using the residuals of a regression of log-mass on log-SVL (Moore et al., 2001; Schulte-Hostedde et al., 2005). To estimate traffic levels, we counted each vehicle that passed during nights spent searching for road snakes. Vehicles were counted only if they were driving on a road within the study site(s) between the hours of 2100 and 0200. If a road snake was captured, any vehicle(s) that passed while the snake was being processed were also included in the tally.

Snakes were bled from the caudal vein using a heparin-treated syringe equipped with a 25 gauge needle. We obtained blood as quickly as possible following safe capture (baseline CORT) and again 30 min after capture (stressed CORT). Snakes were placed in a pillow-case and confined in a cooler at ambient temperature following baseline blood draw. Because snakes attempted to avoid capture and restraint, and because blood draw often took several minutes, the time between capture and baseline sample (initial bleed time; mean ± SE: 5.59 ± 0.30; range: 2.67–8.00 min), and between initial sighting and stressed sample extraction (second bleed time; mean ± SE: 38.55 ± 0.91), were variable. Actual blood draw times were recorded to account for this variability. There