PHYSIOLOGICAL AND BEHAVIORAL EFFECTS OF HARASSMENT ON WILD WESTERN DIAMOND-BACKED RATTLESNAKES

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ABSTRACT

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KARINA Y. COCKS

Human-rattlesnake interactions are becoming more common as human development increases. Informed management of these interactions is crucial to prevent snakebite in humans while preserving rattlesnakes' roles in their ecosystems. The objective of this study was to understand how rattlesnakes respond to the harassment followed by removal to a new area to determine whether providing a minimal stressor to the snakes will minimize human-snake interactions. This work may inform future management strategies that can decrease the frequency of human-rattlesnake interactions. I investigated the effects of a novel harassment method on the plasma corticosterone (CORT) levels in wild Western Diamond-backed Rattlesnakes (*Crotalus atrox*) in Arizona. I also investigated the behavioral, spatial, and physiological effects of repeated instances of the novel harassment method coupled with short distance translocation (SDT) in five telemetered adult male *C. atrox* at Tuzigoot National Monument.. Corticosterone levels in snakes that experienced harassment were higher than baseline CORT levels. Snakes that were treated with adrenocorticotropin (ACTH), which is meant to elicit a "maximum" CORT response, did not differ in plasma CORT levels from snakes that experienced harassment. At Tuzigoot National Monument, three snakes were subjected to harassment and SDT ("treatment"), and two snakes received SDT without harassment ("control"). While sample sizes were too small to allow for statistical analyses, patterns in the data suggested that: 1) snakes that experienced harassment and SDT had smaller activity ranges than snakes that only experienced SDT; 2) there was no difference in the distance moved per day excluding treatment days between the two groups; 3) post-treatment movement (flight) distances did not differ between the two groups; 4) average 24-hour Overall Dynamic Body Acceleration (ODBA) was not affected by treatment; 5) there was no difference in body temperatures between groups; 6) there was no difference in defensive behavior between groups; and 7) there was no difference in post-harassment CORT levels between groups. My findings indicate that Western Diamond-backed Rattlesnakes responded to this novel harassment method with a high stress response. However, there was little evidence that spatial ecology, body temperatures, and behaviors changed as a result of harassment coupled with SDT. More research and additional data analyses are needed to determine if repeated instances of this harassment method coupled with SDT could lead to human avoidance and learning in rattlesnakes and if this method could be used as a management strategy to decrease the likelihood of human-rattlesnake interactions.

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PREFACE

This thesis includes three chapters that follow Northern Arizona University thesis requirements for formatting. Chapter One includes a literature review of the significance of human-rattlesnake interactions, current forms of rattlesnake management, the relationship between stress and learning in reptiles and other animals, and the impact of human disturbance on defensive behavior in rattlesnakes. Chapter Two investigates the effects of a novel stressor on plasma corticosterone (CORT) levels in wild Western Diamond-backed Rattlesnakes. I investigated how plasma CORT levels post-harassment differed from unmanipulated baseline CORT levels and adrenocorticotropin (ACTH) manipulated CORT levels. The objective of this research was to determine if the novel harassment method used in this study successfully elevates plasma CORT levels in rattlesnakes. Chapter Three investigates the spatial, behavioral, and physiological effects of repeated instances of the harassment method coupled with short distance translocation (SDT). The objective of this study, which builds on the results presented in Chapter 2, was to inform management strategies that can be used to decrease human-rattlesnake interactions in areas where encounters with rattlesnakes are not avoidable.

CHAPTER 1

RATTLESNAKE MANAGEMENT, BEHAVIOR, AND STRESS RESPONSE IN A CONSERVATION CONTEXT

Introduction

Human disturbance, including land development, is steadily increasing the occurrence of negative human-wildlife interactions (Lowry et al. 2013, Found and St. Clair 2018). Land utilization and development is increasing in desert habitats, making human-rattlesnake interactions much more common (Sullivan et al. 2017). An increase in human-rattlesnake interactions can lead to an increased risk of snakebite in humans (Dart et al. 1992, Tokish et al. 2001, Campbell and Lamar 2004, Corneille et al. 2006). According to the World Health Organization, venomous snakebite is a “potentially life-threatening disease caused by toxins in the bite of a venomous snake” (WHO; accessed 11/06/2020); thus snakebite is recognized as a global public health issue that can cause devastating complications, long-term morbidity, and even death in humans (Chippaux 1998, Kasturiratne et al. 2008). Interactions with humans can also cause negative impacts on rattlesnakes such as persecution (Greene and Campbell 1992; Greene 1997, 2013; Nowak and Greene 2016) and disease (Clark et al. 2011, Nowak and Greene 2016). Rattlesnakes are important organisms in the ecosystems they inhabit by serving as predators (Klauber 1972, Sun et al. 2001, Nowak et al. 2008) as well as assisting seed dispersal and germination (Engel 1997, Reiserer et al. 2018). Decreasing human-rattlesnake interactions by managing the presence of rattlesnakes in human-modified environments is critical in order to decrease snakebite and preserve rattlesnakes’ roles in ecosystems.

Areas where rattlesnake-human interaction management is essential include public lands, such as national parks and monuments. National parks must remain loyal to their purpose, which is “to conserve the scenery and natural and historic objects and the wildlife therein and to provide for the enjoyment of the same in such manner and by such means as will leave them unimpaired for the enjoyment of future generations” (National Park Service 1980). To conserve rattlesnakes in national parks while keeping visitor safety in mind, rattlesnakes must be managed with care in these areas. The use of habitat modification in urban areas can be successful in reducing the chance of interactions (Nowak 2009, Sullivan et al. 2014); however, this is not applicable in natural areas such as national parks and monuments where long-term habitat modification is often not permitted.

Nowak and van Riper (1999) found that many national parks and monuments used translocation as the primary method for management, however, this method has proven fatal to rattlesnakes (Reinert and Rupert 1999, Nowak et al. 2002, Brown et al. 2011) and is often ineffective (Sullivan et al. 2014). Long distance translocation (LDT) outside of the home range results in increased mortality rates and abnormal movement patterns and spatial ecology (Butler et al. 2005, reviewed in Sullivan et al. 2014, Wolfe et al. 2018), possibly because it increases stress (Heiken et al. 2016). Short distance translocation (SDT) within the home range is likely a widely used management method (Brown et al. 2009, Sullivan et al. 2014, Nowak and Greene 2016); it has proven to be better for overall snake health and survival but is often ineffective in altering human-snake interactions (Hardy et al. 2001, Brown et al. 2009; but see Sealy 2002, Peterson and Sealy 2021). Since translocation alone has proven to be problematic, another management strategy is needed to decrease rattlesnake-human interactions.

Many studies have shown that reptiles will change their behaviors in response to stress induced by novel stressors and predators (Thaker et al. 2009, 2010; Trompeter and Langkilde 2011; Herr et al. 2017; Lauger 2019). Organisms respond to stressful stimuli by producing adrenal hormones such as the glucocorticoid corticosterone (CORT) (Sapolsky et al. 2000). Increased CORT levels (i.e., a CORT response) have resulted in behavioral changes in lizards (Thaker et al. 2009, 2010; Trompeter and Langkilde 2011), including responding quickly to a stimulus, hiding longer, displaying more (defensive displays) towards the predator, increased flight initiation distance, and decreased hiding during subsequent encounters. Thaker et al. (2010) found that there was a relationship between CORT elevation and recollection and learning in lizards that were faced with a novel predator. Lizards that had experimentally suppressed CORT production during encounters with a novel predator had limited learning and less recall during future encounters with the predator (Thaker et al. 2010). Trompeter and Langkilde (2011) found that lizards changed their anti-predator behavior from hiding under objects, which is needed to avoid natural predators such as birds and snakes, to climbing up objects to avoid non-native fire ants. The results from these studies show the important relationship between CORT elevation and adaptation to novel predators and stressors in reptiles. The relationship between elevated CORT responses and learned anti-predator behaviors could be used to inform management strategies for decreasing human-rattlesnake interactions by eliciting new or changed behaviors in rattlesnakes to avoid humans.

One method that can be used to understand stress response to a particular negative stimulus is the administration of an adrenocorticotropic hormone (ACTH) challenge. ACTH is a pituitary hormone that causes the adrenal glands to secrete maximal levels of glucocorticoids (Silvestre 2014). In some studies, ACTH challenges are used as assay validations to confirm that elevated CORT caused by the ACTH injection is also seen in the CORT levels detected in the study samples (i.e., plasma or feces; Frigerio 2004, Young et al. 2004, Lentini 2008). Other studies use ACTH to induce a CORT response to compare maximal CORT responses across different contexts (i.e., seasonality, sex, life history, etc.; Mahmoud et al. 1996, Romero and Wingfield 1998, Dayger and Lutterschmidt 2016). Along with determination of baseline plasma CORT levels, ACTH manipulated CORT responses allow the comparison of CORT responses to a particular stimulus to be compared to baseline and "maximum" CORT responses.

Pitvipers often show no change in defensive behaviors in response to elevated CORT levels. Claunch et al. (2017) concluded that Northern Pacific Rattlesnakes (*Crotalus oreganus*) did not show an increase in defensive behavior in response to elevated plasma CORT levels. Herr et al. (2017) also concluded that Cottonmouths (*Agkistrodon piscivorous*) did not show any relation between post-harassment stress and an increase in defensive behavior. However, some species of pitvipers have shown an increased CORT response to stressors including capture and handling (Dunlap and Wingfield 1995, Moore et al. 2000, Schuett et al. 2004, Dickens et al. 2010, Holding et al. 2014a) and translocation (Holding et al. 2014a, Heiken et al 2016). An increase in CORT in response to stress has been shown to alter spatial ecology in snakes experiencing both SDT (Holding et al. 2014a) and LDT (Heiken et al. 2016). Holding et al. (2014a) inflicted stress on rattlesnakes (*Crotalus oreganus oreganus*) during a 6-week period using SDT and capture and handling, which successfully increased CORT levels. The study subjects showed no evidence of changes in testosterone levels, stress reactivity, behaviors, or body condition in response to weekly capture and short distance translocation. They did, however, document a CORT response to the stressors in all snakes and an increase in activity range sizes. They also found that baseline CORT in treatment snakes remained the same from the beginning to the end of the experiment. The authors conclude that short-distance translocation and capture/handling in rattlesnakes does not cause rattlesnakes chronic stress, but rather multiple instances of acute CORT elevation (Holding et al. 2014a). Ideally, using a stress regime similar to that of Holding et al. (2014a) on rattlesnakes over a longer period of time (i.e., 6 months) could produce behavioral, spatial, and hormonal responses, and result in an aversive behavioral response.

The use of a repeated stressor or harassment coupled with SDT over a 6-month period could be a promising alternative to less successful rattlesnake management strategies. Rattlesnakes have been shown to change their behavior, spatial ecology, and physiological responses in response to human interactions (Schuett et al. 2004, Heiken et al. 2016, Herr et al. 2017). Inflicting a negative stimulus on nuisance animals such as bears (Mazur 2010, Rauer et al. 2003) and elk (Kloppers et al. 2005, Found et al. 2018, Found and St. Claire 2018) has successfully changed behaviors in these animals. Found et al. (2018) determined that conditioning a response through a negative stimulus worked best at intermediate frequencies for elk that had habituated to humans. Therefore, repeated instances of harassment coupled with short distance translocation could be used to deter rattlesnakes from certain high-traffic public use areas (i.e., around dwellings, along trails) within areas such as national parks and monuments where other management strategies such as habitat modification are unavailable. A better understanding of rattlesnake responses to repeated harassment could help inform management strategies to decrease human-rattlesnake interactions in these protected areas.

Montezuma Castle National Monument (MOCA) is a long-term monitoring location for *Crotalus atrox* (Western Diamond-backed Rattlesnake), and *C. molossus* (Northern Black-tailed Rattlesnake; Nowak and van Riper III 1999, Amarello et al. 2010, Nowak and Greene 2016), located in Camp Verde, Yavapai County, Arizona. The site contains a 0.3-mile paved trail that allows visitors to walk in a loop through the riparian areas just north of Beaver Creek and against the south-facing cliff containing the ancient dwellings of the Sinagua people. This site averaged approximately 405,337 visitors per year between 2011 and 2021 (NPS.gov; accessed 4, January 2023). It is also well documented that this site is used by *C. atrox* (Nowak and van Riper III 1999, Amarello et al. 2010, Nowak and Greene 2016, Cocks et al. 2019, E. Nowak, unpubl. data), which can be noted by visitors reading the extensive signage posted by the monument staff to alert them to watch for rattlesnakes.

Tuzigoot National Monument (TUZI) is also a long-term monitoring location for *C. atrox* (Nowak 2005, E. Nowak unpubl. data) located in Clarkdale, Arizona. The site contains a 0.4-mile paved trail that takes visitors around pueblos and rock walls that are used by rattlesnakes for hibernation and retreats (Nowak and van Riper III 1999, Nowak 2005, Nowak and Greene 2016, E. Nowak unpubl. data). The remainder of the monument consists of an unpaved trail system that encompasses a large freshwater marsh and runs along the Verde River. This site averaged approximately 97,053 visitors annually between 2011 and 2021 (NPS.gov; accessed 4, January 2023).

Tuzigoot and Montezuma Castle National Monuments are heavily used by both rattlesnakes and humans. Humans and rattlesnakes often use the same paths, structures, and routes in these monuments, making the possibility of human-rattlesnake interactions high (Nowak and Greene 2016). These monuments provide unique field study sites for rattlesnake-human interactions due to long-term monitoring, history, and heavy, well-documented human recreation.

In contrast, the study site located in Apache Junction is not a long-term monitoring site and has been used in one previous study (Brusch and DeNardo 2017), and it receives significantly less human foot traffic than the NPS sites. The Apache Junction site is a large wash with surrounding native vegetation and is bordered on all sides by public roads and private property. There are indicators of human presence and activity such as powerlines, maintenance roads, and small hiking trails. However, this site does not experience the extent of public visitation that the NPS sites do. This field site serves as a comparison to the NPS sites due to less human disturbance and a drier, hotter environment.

This project aims to explore behavioral patterns exhibited by adult Western Diamond-backed Rattlesnakes (*Crotalus atrox*) in response to repeated harassment. This project has two goals: 1) observe plasma corticosterone levels as a proxy for stress in harassed adult *C. atrox* and determine how that level compares to baseline plasma corticosterone as well as to maximum corticosterone levels manipulated with adrenocorticotropin (ACTH); and 2) determine whether telemetered adult male *C. atrox* exhibit differences in spatial ecology and defensive behavior after standardized harassment and short-distance translocation over a six-month period. I hypothesized that harassed snakes would show an increase in plasma CORT response compared to baseline, but not to maximal levels. I predicted that snakes that were harassed would have lower concentrations of plasma CORT compared to plasma CORT after an ACTH challenge and higher concentrations of plasma CORT compared to the baseline plasma CORT of the snakes. I hypothesized that harassed snakes which experienced short-distance translocation would show a difference in spatial ecology and defensive behavior compared to snakes that were not harassed but experienced short-distance translocation. I predicted that snakes experiencing harassment would have larger activity ranges, increased frequency of movements, movement away from site of harassment, and no difference in defensive behavior compared to snakes that were not harassed.

Synthesis

In Chapter Two, I detail results from the comparison of baseline, harassed, and ACTH manipulated CORT levels taken from free-ranging *C. atrox*. I found that unmanipulated baseline CORT levels were lower than post-harassment and post-ACTH CORT levels which is consistent with previous studies (Mahmoud et al. 1996, Schuett et al. 2004, Langkilde and Shine 2006, Klukowski 2011, Holding et al. 2014a, Dayger and Lutterschmidt 2016, Moeller et al. 2017). I also found that there was no difference in CORT response between males and females. Although other studies have found that there is a sex difference in CORT response (Lance et al. 2001, Matheis et al. 2001, Moore and Jessop 2003), differences in sex responses are also dependent on environmental factors, body condition, and individual variation (Jessop et al. 2000, Romero and Wikelski 2001, Capehart et al. 2016, Dayger and Lutterschmidt 2016, Claunch et al. 2017), which were not examined in this study. To further our understanding of the different physiological responses to this harassment, more studies must be done to assess the impact of sex differences, body condition, and environmental factors on the plasma CORT responses to the harassment method in this study.

In Chapter Three, I detail results from the investigation of spatial ecology, behavior, and physiological effects of repeated instances of harassment coupled with short distance translocation (SDT). Three snakes were subjected to harassment and SDT (“treatment”), and two snakes received SDT without harassment (“control”). My data analyses were limited by small sample sizes; it would be informative to repeat this research with larger sample sizes. Patterns in the data suggested that snakes that experienced harassment and SDT had larger activity ranges than snakes that only experienced SDT. This is similar to the results of Holding et al. (2014a) who found that SDT increased activity range size. I found that the distance moved per day not including treatment days did not differ between treatment and control snakes, which was also seen by Holding et al. (2014a). My research was the first to investigate the distance moved per day in response to harassment and SDT. Post-treatment movement (flight) distances did not differ between groups and although some studies have found that rattlesnakes will return to their site of capture after experiencing SDT (Hardy et al. 2001, Brown et al. 2009, Holding et al. 2014a), post-treatment flight distances in response to translocation or harassment have not been investigated. Overall Dynamic Body Acceleration (ODBA) was variable among each snake and was not impacted by harassment and SDT or SDT alone. However, ODBA is used as a proxy for movement patterns and high values of ODBA can be inaccurately attributed to high frequency movements (Tatler et al. 2018). Body temperature did not differ between groups or between individuals, which is consistent with other studies (Roe et al. 2010, Heiken 2013, Holding et al. 2014b). There was no difference in defensive behavior among the treatment and control groups, which is similar to the results of Claunch et al. (2017) and Herr et al. (2017). There was a suggestion that control snakes had a higher plasma CORT concentration post-harassment at the end of the study than treatment snakes, but to fully understand the long-term effects of this harassment on plasma CORT, changes in baseline CORT levels should be investigated following Holding et al. (2014a) and Heiken et al. (2016).

LITERATURE CITED

Amarello, M., E.M. Nowak, E.N. Taylor, G.W. Schuett, R.A. Repp, P.C. Rosen, and D.L. Hardy, Sr. 2010. Potential environmental influences on variation in body size and sexual dimorphism among Arizona populations of the western diamond-backed rattlesnake (*Crotalus atrox*). Journal of Arid Environments 74: 1443-1449.

Brown, J., C. Bishop, and R. Brooks. 2009. Effectiveness of short-distance translocation and its effects on western rattlesnakes. Journal of Wildlife Management 73: 419-425.

Brown, T.K., J.M. Lemm, J. Montagne, J.A. Tracey, and A.C. Alberts. 2011. Spatial ecology, habitat use, and survivorship of resident and translocated Red Diamond Rattlesnakes (*Crotalus ruber*). *In* W. K. Hayes, K. R. Beaman, M.D. Cardwell, and S. P. Bush (eds.), The Biology of the Rattlesnakes, pp. 377-394. Loma Linda University Press, Loma Linda, CA.

Brusch, G.A., and D.F. DeNardo. 2017. When less means more: dehydration improves innate immunity in rattlesnakes. Journal of Experimental Biology 220(12): 2287-2295.

Butler, H., B. Malone, and N. Clemann. 2005. The effects of translocation on the spatial ecology of tiger snakes (*Notechis scutuatus*) in a suburban landscape. Wildlife Research 32: 165-171.

Campbell, J.A., and W.W. Lamar. 2004. The venomous reptiles of the Western Hemisphere, Volume 2. Cornell University Press (Comstock Publishing Associates), Ithaca, New York, USA.

Capehart, G.D., C. Escallon, B.J. Vernasco, I.T. Moore, and E.N. Taylor. 2016. No drought about it: effects of supplemental hydration on the ecology, behavior, and physiology of free-ranging rattlesnakes. Journal of Arid Environments 134: 79-86.

Chippaux, J.P. 1998. Snake-bites: appraisal of the global situation. Bulletin of the World Health Organization 76: 515-524.

Clark, R.W., M.N. Marchand, B.J. Clifford, R. Stechert, and S. Stephens. 2011. Decline of an isolated Timber Rattlesnake (*Crotalus horridus*) population: interactions between climate change, disease, and loss of genetic diversity. Biological Conservation 144: 886-891.

Claunch, N.M., J.A. Frazier, C. Escallon, B.J. Vernasco, I.T. Moore, and E.N. Taylor. 2017. Physiological and behavioral effects of exogenous corticosterone in a free-ranging ectotherm. General and Comparative Endocrinology 248: 87-96.

Cocks, K, E.M. Nowak, and C. Calvo. 2019. Does anthropogenic disturbance influence defensive behavior of *Crotalus atrox* in national monuments. Biology of Pitvipers Symposium 3. Rodeo, NM.

Corneille, M.G., S. Larson, R.M. Stewart, D. Dent, J.G. Myers, P.P. Lopez, M.J. McFarland, and S.M. Cohn. 2006. A large single-center experience with treatment patients with crotalid envenomations: outcomes with and evolution of antivenin therapy. American Journal of Surgery 192: 848-852.

Dart, R. C., J.T. McNally, D. W. Spaite, and R. Gustafson. 1992. The sequelae of pitviper poisoning in the United States. Pp. 395-404 *In* J.A. Campbell and E.D. Brodie, Jr. (Eds.), Biology of the Pitvipers, Selva, Tyler, Texas.

Dayger, C.A., and D. I. Lutterschmidt. 2016. Seasonal and sex differences in responsiveness to adrenocorticotropic hormone contribute to stress response plasticity in red-sided garter snakes (*Thamnophis sirtalis parietalis*). Journal of Experimental Biology 219: 1022-1030.

Dickens, M.J., D.J. Delehanty, and L.M. Romero. 2010. Stress: an inevitable component of animal translocation. Biological Conservation 143: 1329-1341.

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

Engel, T.R. 1997. Seed dispersal and plant regeneration by snakes? Ecotropica 3: 33-41.

Found, R., and C.C. St. Claire. 2018. Personality influences wildlife responses to aversive conditioning. The Journal of Wildlife Management 82:747-755.

Found, R., E.L. Kloppers, T.E. Hurd, and C.C. St. Claire. 2018. Intermediate frequency of aversive conditioning best restores wariness in habituated elk (*Cercus canadensis*). PLoS One 13: e0199216. https://doi.org/10.1371/journal.pone.0199216.

Frigerio, D., J. Dittami, E. Möstl, and K. Kotrschal. 2004. Excreted corticosterone metabolites co-vary with ambient temperature and air pressure in male Greylag geese (*Anser anser*). General and Comparative Endocrinology 137:29-36.

Greene, H.W. 1997. Snakes. The Evolution of Mystery in Nature. University of California Press, Berkley, California.

Greene, H.W. 2013. Tracks and Shadows. Field Biology as Art. University of California Press, Berkeley, California.

Greene, H.W. and J.A. Campbell. 1992. The future of pitvipers. Pp. 421-427 *In* J.A. Campbell and ED. Brodie (Eds.), Biology of the Pitvipers. Selva Press, Tyler, Texas.

Hardy, D.L., Sr., H.W. Greene, B. Tomberlin, and M. Webster. 2001. Relocation of nuisance rattlesnakes: problems using short-distance translocation in a small rural community. Pp. 44-45 *In* W.L. Halvorson and B.S. Gebow (Eds.), Creative Cooperation in Resource Management: Third Conference on Research and Resource Management in the Southwest Desert, Extended Abstracts. USGS Sonoran Desert Field Station, University of Arizona, Tucson, Arizona.

Heiken, K.H. 2013. The behavioral and physiological effects of long-distance translocation on Western Rattlesnakes (*Crotalus oreganus*). Unpublished Master's Thesis, California Polytechnic State University, San Luis Obispo, California, USA.

Heiken, K.H., G.A. Brusch, S. Gartland, C. Escallon, I.T. Moore, and E.N. Taylor. 2016. Effects of long distance translocation on corticosterone and testosterone levels in male rattlesnakes. 2016. General and Comparative Endocrinology 237: 27-33.

Herr, M.W., S.P. Graham, and T. Langkilde. 2017. Stressed snakes strike first: Hormone levels and defensive behavior in free ranging cottonmouths (*Agkistrodon piscivorus*). General and Comparative Endocrinology 243: 89-95.

Holding, M.L, D.A.S. Owen, and E.N. Taylor. 2014a. Evaluating the thermal effects of translocation in a large-bodied pitviper. JEZ-A Ecological and Integrative Physiology 321(8): 442-449.

Holding, M.L., J.A. Frazier, S.W. Dorr, S.N. Henningsen, I.T. Moore, and E.N. Taylor. 2014b. Physiological and behavioral effects of repeated handling and short-distance translocation on free-ranging Northern Pacific Rattlesnakes (*Crotalus oreganus oreganus*). Journal of Herpetology 48: 233-239.

Jessop, T.S., M. Hamann, M.A. Read, and C.J. Limpus. 2000. Evidence for a hormonal tactic maximizing green turtle reproduction in response to a pervasive ecological stressor. General and Comparative Endocrinology 118: 407-417.

Kasturiratne, A., R. Wickremasinghe, N. de Silva, N.K. Gunawardena, A. Pathmeswaran, R. Premaratna, L. Savioli, D.G. Lalloo, and H.J. de Silva. 2008. The global burden of snakebite: a literature analysis and modelling based on regional estimates of envenoming and deaths. PLoS Medicine 5: 1591-1604.

Klauber, L.M. 1972. Rattlesnakes: Their Habits, Life Histories, and Influence on Mankind, 2 Vols., 2nd ed. University of California Press, Berkeley and Los Angeles.

Kloppers, E.L., C. C. St. Clair, and T.E. Hurd. 2005. Predator-resembling aversive conditioning for managing habituated wildlife. Ecology and Society 10(1): 31.

Klukowski, M. 2011. Effects of breeding season, testosterone, and ACTH on the corticosterone response of free-ranging male fence lizards (*Sceloporus undulatus*). General and Comparative Endocrinology 173(2): 295-302.

Lance, V.A., J.S. Grumbles, and D.C. Rostal. 2001. Sex differences in plasma corticosterone in desert tortoises, *Gopherus agassizii*, during the reproductive cycle. Journal of Experimental Zoology 289: 285-289.

Langkilde, T. and R. Shine. 2006. How much stress do researchers inflict on their study animals? A case study using a scincid lizard, *Eulamprus heatwolei*. Journal of Experimental Biology 209: 1035-1043.

Lauger, K.K. 2019. Stress Physiology, Behavior, and Growth of Federally Threatened Narrow-headed Gartersnakes in Captivity. Unpublished Master's Thesis, Northern Arizona University, Flagstaff, Arizona, USA.

Lentini, A.M. 2008. Investigating behavioural and physiological effects of surgically implanted transmitters on Massasauga rattlesnakes. Unpublished Doctoral dissertation, York University, Toronto, Ontario, Canada.

Lowry, H., A. Lill, and B.B. M. Wong. 2013. Behavioural responses of wildlife to urban environments. Biological Reviews 88: 537-549.

Mahmoud, I.Y, K. Vliet, L.J. Guillette Jr., and J.L. Plude. 1996. Effect of stress and ACTH$_{1\text{-}24}$ on hormonal levels in male alligators, *Alligator mississippiensis*. Comparative Biochemistry and Physiology Part A: Physiology 115(1): 57-62.

Mathies, T., T.A. Felix, and V.A. Lance. 2001. Effects of trapping and subsequent short-term confinement stress on plasma corticosterone in the brown treesnake (*Boiga irregularis*) on Guam. General and Comparative Endocrinology 124: 106-114.

Mazur, R.L. 2010. Does aversive conditioning reduce human-black bear conflict? Journal of Wildlife Management 74: 48-54.

Moeller, K.T., G. Demare, S. Davies, and D.F. DeNardo. 2017. Dehydration enhances multiple physiological defense mechanisms in a desert lizard, *Heloderma suspectum*. Journal of Experimental Biology 220: 2166-2174.

Moore, I. T., J.P. Lerner, D.T. Lerner, R.T. Mason. 2000. Relationships between annual cycles of testosterone, corticosterone, and body condition in male red-spotted garter snakes, *Thamnophis sirtalis concinnu.* Physiological and Biochemical Zoology 73: 307-312.

Moore, I.T. and T.S. Jessop. 2003. Stress, reproduction, and adrenocortical modulation in amphibians and reptiles. Hormones and Behavior 43: 39-17.

National Park Service. 1980. United States Codes: Department of the Interior: National Park Service Organic Act. Title 16, Subchapter 1.

National Park Service. 2022. Annual visitation by park. https://irma.nps.gov/Stats/SSRSReports/Park%20Specific%20Reports/Annual%20Park%20Recreation%20Visitation%20(1904%20-%20Last%20Calendar%20Year)?Park=MOCA. Accessed 4, January 2023.

National Park Service. 2022. Annual visitation by park. https://irma.nps.gov/Stats/SSRSReports/Park%20Specific%20Reports/Annual%20Park%20Recreation%20Visitation%20(1904%20-%20Last%20Calendar%20Year)?Park=TUZI; Accessed 4, January 2023.

Nowak, E.M. 2005. Movement patterns and life history of wester diamond-backed rattlesnakes (*Crotalus atrox*) at Tuzigoot National Monument, Arizona. Pp. 235-274 *In* C. van Riper

III and D. Mattson, (Eds.), The Colorado Plateau II: Biophysical, socioeconomic, and cultural research. University of Arizona Press, Tucson, Arizona.

Nowak, E.M. 2009. Ecology and management of venomous reptilian predators. Ph. D. Dissertation, Northern Arizona University, Flagstaff, Arizona.

Nowak, E.M. and C. van Riper III. 1999. Effects and effectiveness of rattlesnake relocation at Montezuma Castle National Monument. U.S. Geological Survey/FRESC Report Series USGS/FRESC/COPL/1999/17.

Nowak, E.M. and H.W. Greene. 2016. Rattlesnake Conservation in the 21st Century. Pp 413-458 *In* G.W. Schuett, M.J. Feldner, C.F. Smith, and R.S. Reiserer (Eds.), Rattlesnakes of Arizona, Volume 2: Conservation, Behavior, Venom, and Evolution. ECO Herpetological Publishing, Rodeo, New Mexico.

Nowak, E.M., T. Hare, and J. McNally. 2002. Management of "nuisance" vipers: effects of translocation on western-diamond-backed rattlesnakes (*Crotalus atrox*). Biology of the Vipers 34: 533-560.

Nowak, E.M., T.C. Theimer, and G.W. Schuett. 2008. Functional and numerical responses of predators: Where do vipers fit in the traditional paradigms? Biological Review 83: 601-620.

Peterson, C.E., and J.B. Sealy. 2021. Threats and Conservation Status. Pp. 81-98 *In* Breisch, A.R., W.H. Martin, J.B. Sealy, C.E. Peterson, and E. Possardt (Eds). The Timber Rattlesnake: Life History, Distribution, Status, and Conservation Action Plan. Partners in Amphibian and Reptile Conservation Technical Publication CAP-1. Published by the Amphibian and Reptile Conservancy, Nashville, Tennessee.

Rauer, G., P. Kaczensky, and F. Knauer. 2003. Experiences with aversive conditioning of habituated brown bears in Austria and other European countries. Ursus 14: 215-224.

Reinert, H.K., and R.R. Rupert. 1999. Impacts of translocation on behavior and survival of timber rattlesnakes, *Crotalus horridus*. Journal of Herpetology 33: 45-61.

Reiserer, R.S., G.W. Schuett, and H.W. Greene. 2018. Seed ingestion and germination in rattlesnakes: overlooked agents of rescue and secondary dispersal. Proceedings of the Royal Society B: Biological Sciences 285: 20172755.

Roe, J.H., M.R. Frank, S.E. Gibson, O. Attum, and B.A. Kingsbury. 2010. No place like home: an experimental comparison of reintroduction strategies using snakes. Journal of Applied Ecology 47(6): 1253-1261.

Romero, L., and M. Wikelski. 2001. Corticosterone levels predict survival probabilities of Galapagos marine iguanas during El Nino events. Proceedings of the National Academy of Sciences of the United States of America 13: 7366-7370.

Romero, L.M., and J.C. Wingfield. 1998. Seasonal changes in adrenal sensitivity alter corticosterone levels in Gambel's White-Crowned Sparrows (*Zonotrichia leucophrys gambelii*). Comparative Biochemistry and Physiology 199C: 31-36.

Sapolsky, R.M., L.M. Romero, and A.U. Munck. 2000. How do glucocoirticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocrine Reviews 21: 55-89.

Schuett, G.W., E.N. Taylor, E.A. Van Kirk, and W.J. Murdoch. 2004. Handling stress and plasma corticosterone levels in captive male Western Diamond-backed Rattlesnakes (*Crotalus atrox*). Herpetological Review 35: 229-233.

Sealy, J.B. 2002. Ecology and behavior of the Timber Rattlesnake (*Crotalus horridus*) in the upper piedmont of North Carolina: Identified threats and conservation recommendations. Biology of the Vipers 2002: 561-578.

Silvestre, A.M. 2014. How to assess stress in reptiles. Journal of Exotic Pet Medicine 23: 240-243.

Sullivan, B.K., D.J. Leavitt, and K.O. Sullivan. 2017. Snake communities on the urban fringe in the Sonoran Desert: influences on species richness and abundance. Urban Ecosystems 20: 199-206.

Sullivan, B.K., E.M. Nowak, and M.A. Kwiatkowski. 2014. Mitigation translocation of nuisance herpetofauna: can it be improved? Conservation Biology 29: 12-18.

Sun, L., R. Shine, Z. Debi, T. Zhengren. 2001. Biotic and abiotic influences on activity patterns of insular pit-vipers (*Gloydius shedaoensis*, Viperidae) from north-eastern China. Biological Conservation 97: 387-398.

Tatler, J., P. Cassey, and T.A.A. Prowse. 2018. High accuracy at low frequency: detailed behavioral classification from accelerometer data. Journal of Experimental Biology 221: jeb184085.

Thaker, M., A.T. Vanak, S.L. Lima, and D.K. Hews. 2010. Stress and aversive learning in a wild vertebrate: the role of corticosterone in mediating escape from a novel stressor. The American Naturalist 175: 50-60.

Thaker, M., S. Lima, and D.K. Hews. 2009. Acute corticosterone elevation enhances antipredator behaviors in male tree lizard morphs. Hormones and Behavior 56: 51-57.

Tokish, J.T., J. Benjamin, and F. Walter. 2001. Crotalid envenomation: the southern Arizona experience. Journal of Orthopaedic Trauma 15: 5-9.

Trompeter, W.P., and T. Langkilde. 2011. Invader danger: lizards faced with novel predators exhibit an altered behavioral response to stress. Hormones and Behavior 60: 152-158.

Wolfe, A.K., P.A. Fleming, and P.W. Bateman. 2018. Impacts of translocation on a large urban-adapted venomous snake. Wildlife Research 45: 316-324.

World Health Organization. 2020. Snakebite. https://www.who.int/health-topics/snakebite#tab=tab_1 . Accessed 6, November 2020.

Young, K.M., S.L. Walker, C. Lanthier, W.T. Waddell, S.L. Monfort, J.L. Brown. 2004. Noninvasive monitoring of adrenocortical activity in carnivores by fecal glucocorticoid analyses. General and Comparative Endocrinology 137: 148-165.

CHAPTER 2

COMPARISONS OF BASELINE, HARASSED, AND ADRENOCORTICOTROPIN MANIPULATED PLASMA CORTICOSTERONE SAMPLES FROM FREE-RANGING WESTERN DIAMOND-BACKED RATTLESNAKES

Introduction

Organisms respond to stressful stimuli by increasing glucocorticoids such as cortisol or corticosterone (CORT), which are adrenal cortical hormones (Sapolsky et al. 2000). Measuring CORT concentrations in blood samples is a strategy used as a proxy for assessing the stress response in pitvipers (Lance 1990, Wingfield and Romero 2001, Greenberg 2002, Moore and Jessop 2003). Human influences such as capture and handling (Dunlap and Wingfield 1995, Moore et al. 2000, Schuett et al. 2004, Dickens et al. 2010, Holding et al. 2014a) and translocation (Holding et al. 2014a, Heiken et al. 2016) can increase CORT levels in pitvipers. Other environmental factors can increase CORT levels in reptiles including hydration state (Capehart et al. 2016, Claunch et al. 2017), food availability (Romero and Wikelski 2001), and extreme temperatures (Jessop et al. 2000).

Acute elevations of plasma CORT concentrations are short (i.e., lasting minutes or hours) in duration (Burchfield 1979, Romero 2004). Chronically elevated concentrations of plasma CORT are long (i.e., days or months) in duration (Burchfield 1979, Romero 2004, Sapolsky et al. 2000) and can cause immune deficiency, decreased reproductive success, and decrease body condition (Moynihan 2003, French et al. 2008). Acute CORT elevation is adaptive in avoiding new predators or novel stressors (Bailey et al. 2009, Thaker et al. 2010, Trompeter and Langkilde 2011, Capehart et al. 2016). Thaker et al. (2010) found that lizards manipulated with a corticosterone synthesis blocker did not adapt to attacks by man-made red cube attached to a pole used as a novel stressor. Repeated instances of a negative stimulus could ideally create multiple instances of acute CORT elevations, which aids in predator avoidance (Bailey et al. 2009, Thaker et al. 2010, Trompeter and Langkilde 2011, Capehart et al. 2016) and associations between stimuli (Beylin and Shors 2003). Holding et al. (2014a) used a weekly (7-8 day) time interval to repeatedly inflict a stressor (translocation and capture/handling) on Northern Pacific Rattlesnakes (*Crotalus oreganus oreganus*) over a 6-week period. The study subjects showed no difference in baseline plasma CORT levels between the beginning and end of the study, which presented evidence that the researchers were inflicting multiple instances of acute CORT elevation rather than producing chronic CORT elevation.

One way to validate or induce a CORT response is the use of adrenocorticotropin. Adrenocorticotropin (ACTH) is a hormone that is naturally produced by the pituitary gland, which is stimulated when the HPA axis is initiated by a corticotropin-releasing hormone that is produced by the hypothalamus following a stressful stimulus or threat (Silvestre 2014). The presence of ACTH acts on the adrenal cortex to produce glucocorticoids, including corticosterone (Silvestre 2014). Therefore, an injection of ACTH can cause an animal to secrete “maximum” levels of glucocorticoids (Dayger and Lutterschmidt 2016). In some studies, ACTH is used as a challenge for assay validations (Frigerio 2004, Young et al. 2004, Lentini 2008). In these studies, the samples taken post-ACTH injection are used to confirm that elevated CORT caused by the ACTH injection is reflected in the quantity of CORT found in study samples (i.e., plasma, saliva, feces; Frigerio 2004, Young et al. 2004, Lentini 2008). Other studies have used ACTH to induce a CORT response to compare CORT responses across different contexts (i.e., seasonality, sex, life history; Mahmoud et al. 1996, Romero and Wingfield 1998, Dayger and Lutterschmidt 2016). The use of ACTH to secrete maximum levels of CORT could be used to quantify and validate the level of stress that is being inflicted by an outside stressor such as capture and handling and predator interactions.

Pitvipers have shown an increased CORT response to stressors including capture and handling (Dunlap and Wingfield 1995, Moore et al. 2000, Schuett et al 2004, Dickens et al. 2010, Holding et al. 2014a) and translocation (Holding et al. 2014a, Heiken et al. 2016). Reptiles have the ability to learn and make decisions in response to stress. Multiple studies show that reptiles can modify their behavior in response to stress induced by a novel stressor or predator (Thaker et al. 2010, Trompeter and Langkilde 2011, Krochmal et al. 2018). Learning in rattlesnakes was demonstrated by Krochmal et al. (2018), who conducted a study demonstrating that rattlesnakes were able to decrease their latency in decision making in a thermal maze after only one trial, while non-rattlesnake pitviper relatives showed no modification in latency in decision making after twelve trials through the thermal maze. Lizards have also exhibited an ability to learn and change their behavior in response to novel predators (Trompeter and Langkilde 2011). Some studies, however, have shown that some species of pitvipers do not change their defensive behavior in response to elevated CORT or harassment by humans (Claunch et al. 2017, Herr et al. 2017).

Human-rattlesnake interactions can result in an increased risk of venomous snakebite (Dart et al. 1992, Tokish et al. 2001, Campbell and Lamar 2004, Corneille et al. 2006) in addition to snake mortality from humans (Reinert and Rupert 1999, Sullivan et al. 2014, Nowak and Greene 2016). Rattlesnake populations also serve as important components of their ecosystems by assisting in seed dispersal and germination (Engel 1997, Reiserer et al. 2018) and serving as predators and prey (Klauber 1972, Sun et al. 2001, Nowak et al. 2008). Reducing human-rattlesnake interactions using behavioral responses to CORT elevation through harassment (hazing) by trained personnel could potentially result in a decreased chance of venomous snakebite. Through instances of acute CORT elevation, rattlesnakes could learn to avoid future potentially negative encounters with humans. This stress response could change rattlesnake behaviors to avoid human interactions, while keeping rattlesnakes in place to promote snake conservation and healthy ecosystems. With the use of ACTH injections, we can compare plasma CORT levels in response to harassment to baseline and “maximum” ACTH manipulated CORT levels. Quantifying to what extent this particular stressor raises plasma CORT will help inform potential management strategies to decrease human-rattlesnake interactions.

This project aims to determine if harassment of rattlesnakes could be used as a management tool to discourage rattlesnakes from areas frequented by humans, especially in public spaces. Rattlesnake-human interactions require management in spaces such as national parks and monuments that must conserve the natural land (National Park Service 1980), but also receive heavy human use. Montezuma Castle National Monument (MOCA) and Tuzigoot National Monuments (TUZI) in Yavapai County, Arizona, are long-term monitoring locations for *C. atrox* (Nowak and van Ripper III 1999, Nowak 2005, Amarello et al. 2010, Nowak and Greene 2016, Nowak unpubl. data). Both monuments also experience very heavy human foot traffic with MOCA averaging 405,337 visitors per year and TUZI averaging 97,053 visitors per year (NPS.gov; accessed 4 January 2023). These sites provide a unique opportunity for research on human-rattlesnake interactions due to the heavy use of the park by both humans and rattlesnakes. An additional field site in Apache Junction, Pinal County, Arizona, was included in the study to serve as a comparison to the National Park Service (NPS) field sites. The Apache Junction site had significantly less human foot traffic because it is not a public park and a hotter, drier habitat (i.e., higher annual mean temperature, lower annual rainfall, less available surface water).

I inflicted harassment on adult male free-ranging Western Diamond-backed Rattlesnakes (*Crotalus atrox*) at these three sites by placing rattlesnakes in 5-gallon snake-safe buckets and moving, flipping, lifting, and prodding them with snake tongs for 10 of every 30 seconds during a 30-minute period, and determined if this caused an elevation in plasma CORT levels (Langkilde and Shine 2006, Moeller et al. 2017). I compared the plasma CORT concentrations of snakes treated with harassment to plasma CORT concentrations of non-harassed snakes treated with adrenocorticotropin (ACTH), which shows where the stress response induced by the harassment method lies in comparison to maximum secretory capacity of the adrenal glands. I compared the plasma CORT levels of the snakes treated with harassment to the concentrations of snakes not faced with harassment and not subjected to ACTH challenge to show where the stress response lies in comparison to baseline plasma CORT concentrations. The results of this study will be used to assess whether targeted harassment of rattlesnakes found in human-developed habitats, a novel management strategy, could be used to decrease human-rattlesnake interactions in areas where these interactions are common (i.e., national parks and monuments, residential areas, etc.).

Methods

Study Sites

Montezuma Castle National Monument (MOCA) is located in Camp Verde, Yavapai County, Arizona at 1158 m elevation. The monument is bordered by the United States Forest Service property (Beaver Creek Ranger District) and Interstate Highway I-17. Wet Beaver Creek bisects the monument, and its vegetation is consistent with the characteristics of Sonoran Riparian Deciduous Forest Scrubland (Minckley and Brown 1994). Tuzigoot National Monument (TUZI) is located in Clarkdale, Yavapai County, Arizona at 1036 m elevation. The monument is located approximately 35 kilometers west of Montezuma Castle National Monument (MOCA) and is bordered on the west side by private property, bordered to the north by U.S. Forest Service land, and on the east and south by Arizona State Park land (including the Verde River Greenway). The Verde River runs outside and adjacent to the monument’s borders. The vegetation in this area is consistent with Sonoran Riparian Deciduous Forest Scrubland (Minckley and Brown 1994). Tavasci Marsh, a large freshwater marsh, is located in the monument with vegetation consistent with Sonoran Interior Marshland (Minckley and Brown 1994). Both monuments have vegetation consistent with the Creosote-bush-Crucifixionthorn Series of the Arizona Upland Subdivision of the Sonoran Desertscrub as well as vegetation often seen in the Mojave, Great Basin, and Chihuahuan deserts (Turner and Brown 1994). MOCA averaged approximately 405,337 visitors per year between 2011 and 2021 and TUZI averaged approximately 97,053 visitors per year between 2011 and 2021 (NPS.gov; accessed 4, January 2023). Both MOCA and TUZI are long-term monitoring locations for *C. atrox* (Nowak and van Ripper III 1999, Nowak 2005, Amarello et al. 2010, Nowak and Greene 2016, Nowak unpubl. data).

The Apache Junction site was located in Apache Junction, Pinal County, Arizona at 525 m elevation. The site has been used previously for one other study (Brusch and DeNardo 2017). This site is located on Bureau of Land Management land and is surrounded by both sides by public roads and private property. Its vegetation is consistent with the Paloverde-Cacti-Mixed Scrub Series of the Arizona Upland Subdivision of the Sonoran Desertscrub (Turner and Brown 1994).

Study Snakes

Twenty-five adult (SVL > 70cm, which is above the smallest sexually mature male and female found in *C. atrox* by Taylor and Booth (2016) male and female *C. atrox* were captured during visual encounter surveys and road driving from all three sites between August and September of 2021 and 2022 (Table 1). Visual encounter surveys included looking for snakes by walking through suitable habitat and looking under vegetation, rocky outcrops, and other natural or man-made cover that snakes could use (McDiarmid et al. 1997). Collaboration with NPS staff at MOCA and TUZI also allowed trained staff to opportunistically capture snakes that were seen by visitors or rangers (McDiarmid et al. 1997, Nowak and Greene 2016). Snakes were captured using snake tongs and placed in a modified snake-safe bucket (McDiarmid et al. 1997). Once snakes were captured, they were restrained in clear, plastic tubes for blood drawing, body measurements, and tagging (McDiarmid et al. 1997). Data on body morphology for seventeen snakes captured in 2021 was apparently lost when datasheets were left in clipboard on top of a car. The remaining eight snakes had an SVL range of 70.0 to 99.0 cm and mean 83.0 cm SVL (Table 2-1)

Experimentation (Harassment)

Snakes were opportunistically captured and randomly assigned to one of two groups. Snakes in the first group were immediately bled to determine baseline CORT concentrations. To maximize sample size, the first group was treated with ACTH after baseline CORT concentrations were collected. After baseline CORT concentrations were taken from the first group, the snakes received an intraperitoneal injection of 2 IU/kg following Lentiti (2008). The injection was made by diluting 0.25/mL cosyntropin (0.25 mg/vial from SANDOZ; ACTH equivalent) in a 1:9 saline solution to create a 2.5 IU/mL ACTH concentration. Snakes were each given a 0.8mL/kg injection of ACTH and saline solution. After the injection, snakes were kept in a snake-safe bucket for 1 hour and then bled to assess CORT levels (Dayger and Lutterschmidt 2016).

Snakes in the second group were first harassed, and then bled to quantify post-harassment CORT levels, as a proxy of stress reactivity. Baseline CORT, which would have provided a true quantification of stress reactivity, was not measured in this group of snakes because we wanted the manipulation to duplicate what would be done if harassment was used in efforts to reduce snake-human interactions. A baseline CORT sample would have added additional handling and restraint that would not be a component of an applied effort but could influence snake behavior post-harassment, thus altering the assessment of the effects of harassment. Harassment-induced stress reactivity was instead measured in a separate group of snakes (see Chapter 3). Harassment consisted of placing the snake in a snake-safe 5-gallon bucket and moving, flipping, lifting, and prodding them with snake tongs intermittently for 30 minutes (Langkilde and Shine 2006). I used an interval consistent with Moeller et al. (2017), which is harassing snakes for 10 of every 30 seconds of the 30-minute period. Blood was collected from the snakes immediately following the harassment period.

Sample and Data Collection

To collect a blood sample, the snakes were enticed into clear restraining tubes, and a blood sample was collected (at least 0.1 mL) from the caudal vein using a sterile 1.0 mL syringe with a 25g X 5/8” needle pre-treated with liquid heparin sodium (Sagent^{TM}, 1,000 USP/mL). The seventeen samples with lost data do not have blood draw time associated with the samples; however, baseline blood samples were collected in under ten minutes, and all other samples were collected in under thirty minutes. The remaining eight samples had baseline blood samples collected in six minutes or less, post-ACTH blood samples taken in eleven minutes or less, and post-harassment blood samples taken in eighteen minutes or less. Blood samples were stored in 1.5 mL microtubes at ambient temperatures during fieldwork and kept refrigerated or on ice before being prepared and stored in a -20°C freezer upon return to the lab (usually within 48 hours). After blood collection, but with the snake still in the restraining tube, snout-vent length (SVL) and tail length (TL) were measured (+/- 1 cm) and each snake was sexed. The snakes were weighed in the tube (+/- 1 g) and if snakes were not already micro-chipped at MOCA or TUZI, they were injected with a 10 mm passive integrated transponder (PIT) tag (Biomark, Boise, Idaho). Snakes at the Apache Junction site were marked with black markers on their rattles to prevent recaptures. After processing, the snakes were released at their site of capture.

Sample Preparation

Once blood samples were taken to the lab, they were immediately placed in a centrifuge at 2,000 rpm for 15 minutes. This process separated the plasma from the red blood cells in the sample. The plasma was then extracted using a pipette and stored in microtubes in a -20°C freezer until the samples were ready to be analyzed.

Enzyme Immunoassay of Plasma

Samples were analyzed for CORT using a DetectX® Corticosterone Enzyme Immunoassay Kit (Arbor Assays, K014-H1, Ann Arbor, Michigan). To prepare the samples for the assay, 5µL of plasma was added to a 600 µL tube containing 5 µL of dissociation reagent. This mixture was vortexed and left to incubate at room temperature for at least five minutes. Next, each sample was diluted with 490 µL of diluted assay buffer, resulting in a 100X dilution. Corticosterone standards were prepared following kit instructions with final concentrations ranging from 0 to 5,000 pg/mL. Next, 50 µL of sample or standard (in duplicate) were added to the plate. A pooled plasma sample and control of known hormone concentration was also run in duplicate on every plate. The plates were covered and shaken at room temperature for one hour and were washed in a microplate washer five times using the wash buffer provided by the kit. Then, 100 µL of tri-methyl bromide (TMB) substrate was added to each well and the plate was incubated at room temperature for 30 minutes. After incubation, 50 µL of stop solution was added to each well. The plates were then read at 450 nm at 3, 5, 7, and 10 minutes after stop solution was added using a microplate reader.

Assay Validations

A parallelism validation was performed to confirm that the hormone being measured in the sample was immunologically similar to the CORT standard that was provided in the kit. A pooled sample was diluted (1:2-1:128) and run alongside the assay kit's standard curve. The results were plotted as $\log_{10}$ (relative dose) of CORT by the percentage of the antibody bound (%B/B0). To compare slopes of the diluted pool and the standard curve, an F-test was performed in Prism (Prism 9 for Windows 64-bit, version 9.5.1). Given that the slopes are not statistically different (i.e., the lines were parallel), we can assume that standard curve can be used to proportionally measure hormone concentrations in the snake samples (after Diamandis and Christopoulos 1996).

A test of accuracy was performed to confirm that no other components of the sample interfered with the hormone that was binding in the assay (i.e., matrix effect). Each CORT standard (5000, 2500, 1250, 625, 312.5, 156.25, 78.125 pg/mL) was spiked with pooled diluted plasma and assayed. A linear regression between expected and observed hormone (pg/mL CORT) concentration was then run in GraphPad Prism. For this test, a correlation coefficient greater than 0.95 and a slope between 0.7 and 1.3 defines acceptable accuracy (Hunt et al. 2017b, 2018; Fernandez Ajo et al. 2018; Dillon et al. 2021).

Statistical Analyses

All statistical analyses were performed in R Studio (Posit Software, PBC, version 2022.12.0) unless specified otherwise. Data was tested for normality using Shapiro-Wilk test for normality and tested for homogeneity of variance using the Bartlett's Test (Sonderegger and Buscaglia 2020). Models were created using log-transformed CORT data. The snake, "Stinky" exhibited a lower post-ACTH plasma CORT level than her baseline plasma CORT level. For each data set, linear mixed effects models (Sonderegger 2020) were made using the "lmer" function in the "lme4" package with individual "Snake ID" included as a random effect to account for two CORT samples being taken from each individual snake. Fixed effects included "Group", "Sex", and an interaction between "Group" and "Sex". Multiple models were created with different fixed effects. Analysis of variance (ANOVA) tests were run to compare the models to each other. Once the "best" linear mixed effects model was determined through statistical testing including p-values and Akaike Information Criteria (AIC), Tukey's Post-hoc tests were used to identify significant differences between the groups (Sonderegger 2020). Both p-values and AIC are used to assess the “best” model fit for the data, and the use of either test is appropriate (Halsey 2019, Sonderegger 2020); in this study, both AIC and p-values were used to choose the best model fit for the data.

The dataset had residuals that were not normally distributed due to the effect of the outlier snake “Stinky”. The CORT samples from this snake were re-run to confirm the values and were not removed from the dataset. The dataset was tested for normality and homogeneity of variance using the Shapiro-Wilk test and Bartlett’s test.

Results

Biochemical validations

The results of the accuracy test showed that the observed hormone (CORT) concentration was significantly correlated with the expected hormone (CORT) concentrations (R^2=0.995, slope = 1.235, Figure 2-1; Table 2-2). The parallelism test showed that the slopes of the lines for the pooled sample and the CORT standard were not significantly different (i.e., equivalent/parallel; $F_{1,11}$=0.1348, p=0.7205, Figure 2-2, Table 2-2).

Effects of group and sex on plasma CORT

The Shapiro-Wilk test showed that this data was normally distributed (W=0.9596, p=0.2374), and the Bartlett's test showed equal variances (Bartlett's K^2=4.2577, df=2, p=0.119). There was a significant difference between CORT levels in baseline, harassed, and ACTH groups in a model that did not include sex as a fixed effect. Animals that were harassed or injected with ACTH had elevated CORT levels (X^2=16.756, df = 2, p=0.0002; Table 2-3; Figure 2-3). There was no significant difference between sex across all groups, meaning that males and females showed no difference in CORT response (X^2=0.7084, df = 1, p=0.4; Table 2-3; Figure 2-4). There was no significant difference in the mean CORT in the treatments that is different by sex (i.e., no interaction between group and sex; X^2=0.9052, df = 2, p=0.636; Table 2-3). Although there was visual evidence of an interaction between group and sex (Figure 2-5) the interaction was not statistically significant (X^2=0.9052, df = 2, p=0.636; Table 2-3). There was a significant difference between treatment groups, with sex as a fixed effect (X^2=17.369, df = 2, p=0.0002; Table 2-3; Figure 2-6); this result signifies that there was a difference in mean CORT levels between baseline, harassed, and ACTH groups. Due to the small p-value and small AIC, the model that includes group and sex as fixed effects (Model 4) was determined to be the best model for the data (Halsey 2019, Sonderegger 2020; Table 2-3). The model chosen investigated the effects of treatment group (baseline, post-harassment, and post-ACTH) and sex on the CORT responses of the study animals. A Tukey's Post-hoc analysis on the chosen model showed that there was a significant difference in CORT levels between ACTH and baseline groups (p=0.0017, df=9.63, t=4.599; Figure 2-3; Figure 2-7) and baseline and harassed (p=0.0017, df=27.9, t=-3.632; Figure 2-3); the results showed that both ACTH and harassed groups had higher CORT levels than the baseline group. It also showed that there was no significant difference in CORT levels between ACTH and harassed groups (p=0.7723, df=27.9, t=0.292; Figure 2-3 The results from the Tukey's Post-hoc analysis showed that harassment and ACTH injections raised CORT above unmanipulated baseline CORT levels in the study snakes, meaning that both harassment and ACTH injections successfully raise plasma CORT in rattlesnakes. It also showed that the CORT responses to harassment and ACTH injections were similar, which means that the snakes had a "maximum" response to the harassment strategy.

Discussion

This study was the first, to my knowledge, to investigate the physiological effects of a novel harassment method on the plasma corticosterone (CORT) concentrations in Western Diamond-backed Rattlesnakes (*Crotalus atrox*) with the goal of informing a management strategy to decrease human-rattlesnake interactions. This study was the first to compare CORT concentrations post-harassment to ACTH manipulated CORT concentrations in rattlesnakes. I found that snakes that experienced harassment or received an ACTH injection had higher plasma CORT concentrations than unmanipulated baseline CORT concentrations taken from naïve snakes. I also found that the snakes had similar physiological reactions to both harassment and ACTH injections.

One snake in the study, called “Stinky”, did not exhibit a physiological reaction to an injection of ACTH. Given that repeated analyses of the sample produced consistent CORT levels, this lack of response could have been the results of environmental conditions or individual variation (Moore and Jessop 2003) or simply an error in ACTH administration.

In this study, the baseline and post-ACTH blood samples were taken from the same individual, while the post-harassment sample was taken from naïve snakes that only experienced harassment. Baseline blood samples were not taken from snakes that experienced harassment to remove the influence of capture, handling, and blood draw on the CORT response from these individuals. The purpose of removing these influences was to see the effect of harassment alone on the plasma CORT concentration of snakes and further study the behavioral and physiological changes that this stressor could cause in Chapter 3.

There was no difference between male and female CORT responses. Dayger and Lutterschmidt (2016) found that there was a difference in ACTH response between male and female Red-sided Gartersnakes (*Thamnophis sirtalis parietalis*) gartersnakes during their spring breeding season, but not during the autumn (non-breeding) season. However, *C. atrox* have been noted to breed in both the spring and fall season (Taylor and DeNardo 2004), suggesting that this difference in reproductive strategy may explain the differences between this study and the one in gartersnakes. Although many studies have found that there are sex differences in CORT responses (Lance et al. 2001, Mathies et al. 2001, Moore and Jessop 2003), it should also be noted that seasonality, body condition, and environmental conditions can also influence these differences (Jessop et al. 2000, Romero and Wikelski 2001, Capehart et al. 2016, Dayger and Lutterschmidt 2016, Claunch et al. 2017). Due to major data loss with these samples, we were not able to explore additional environmental effects or the effects of body condition on the CORT responses of these animals. It should also be noted that the inclusion of the outlier snake could have impacted the results for female CORT response. A larger sample size and information about body condition and environmental conditions could better inform potential sex differences in CORT response in this context.

Post-harassment CORT levels and post-ACTH injection CORT levels were higher than unmanipulated baseline CORT levels. This is consistent with studies that have shown that capture, handling, and common field techniques successfully raise plasma CORT levels above baseline in reptiles (Schuett et al. 2004, Langkilde and Shine 2006, Holding et al. 2014a). Moeller et al. (2017) also showed that a similar harassment method successfully raised plasma CORT in captive Gila Monsters (*Heloderma suspectum*). Studies have also shown that ACTH injections successfully raise CORT concentrations above baseline in reptiles (Mahmoud et al. 1996, Klukowski 2011, Dayger and Lutterschmidt 2016). The CORT response to ACTH and harassment, however, were statistically similar. The goal of ACTH injections in this study was to elicit “maximum” CORT responses in the study animals (Dayger and Luttershmidt 2016) and CORT responses to the harassment method in this study were comparable to the ACTH manipulated CORT responses. The similarity between these two responses suggests that the harassment strategy successfully elicits a “maximum” stress response from rattlesnakes. Elevated CORT responses can change anti-predator behaviors in reptiles (Thaker et al. 2009; 2010; Trompeter and Langkilde 2011), so the elevated CORT response to the harassment strategy in this study suggests that this harassment method could be used to change behaviors in rattlesnakes.

According to information reviewed by Trevisi and Bertoni (2008), an ACTH challenge can mimic an acute stress response. Since the post-ACTH and post-harassment CORT responses in this study were similar, we can assume that the post-harassment CORT response was an acute response. Acute elevations of plasma CORT have been shown to be adaptive in reptiles by avoiding novel threats (Bailey et al. 2009, Thaker et al. 2010, Trompeter and Langkilde 2011, Capehart et al. 2016). It is also known that reptiles change their behaviors in response to stress and novel threats (Thaker et al. 2009, 2010; Trompeter and Langkilde 2011; Herr et al. 2017; Lauger 2019). Therefore, the evidence of acute CORT concentrations caused by the harassment method in this study suggests that rattlesnakes could change their behaviors to avoid this novel threat.

Repeated infliction of a stressor has been successful in changing behaviors in bears (Mazur 2010, Rauer et al. 2003), elk (Kloppers et al. 2005, Found et al. 2018, Found and St. Clair 2018), and wolves (Hawley et al. 2009). Repeated instances of the harassment method in this study could potentially change behaviors in rattlesnakes. Alternatively, repeated instances of this acute stressor could eventually cause chronically elevated CORT concentrations, which can negatively impact the snakes through immune deficiency, decreased body condition, and decreased reproductive success (Moynihan 2003, French et al. 2008). Holding et al. (2014a) did not find that weekly instances of handling or translocation caused chronic CORT elevation, however, the study focused on baseline CORT changes during a 2-month period. Further research is needed to investigate the impacts of repeated instances of acute CORT elevation on baseline CORT concentrations over a longer period (i.e., 6 months; see Chapter 3).

Management Implications

Current management strategies for human-rattlesnake interactions include long distance translocation (LDT), short distance translocation (SDT), and habitat modification. However, LDT has proven to be harmful to snake health (Butler et al. 2005, reviewed in Sullivan et al. 2014, Wolfe et al. 2018), SDT is often ineffective in altering human-rattlesnake interactions (Hardy et al. 2001, Brown et al. 2009; but see Sealy 2002 and Peterson and Sealy et al. 2021), and habitat modification is not a viable solution in areas such as national parks and monuments where habitat modification is often not permitted. Following the research of Sealy (2002) and Brown et al. (2009), short distance translocation is a widely used management method for rattlesnakes (Sullivan et al. 2014, Nowak and Greene 2016). It is possible that SDT coupled with the harassment method in this study could lead to a more successful management tool for decreasing instances of human-rattlesnake interactions; however, my sample size was small and further research is needed to understand the behavioral and physiological changes in response to this method.

In Chapter 3, I investigate the spatial, behavioral, and physiological effects of repeated instances of SDT coupled with harassment. Although sample sizes were too small to allow for statistical analysis, patterns in the data suggested that snakes that experienced harassment and

SDT had smaller activity ranges than snakes that only experienced SDT.. The Overall Dynamic Body Acceleration (ODBA) of the snakes did not appear to be affected by harassment and SDT or SDT alone. I also found that there was no difference in the distance moved per day on non-treatment days, post-treatment flight distances, initial defensive behavior, or post-treatment CORT concentrations between groups. The purpose of taking post-harassment CORT concentrations at the end of the study from all snakes was to investigate the difference in physiological reactions between snakes who had experienced harassment versus snakes that had never experienced harassment (i.e., did snakes that experienced harassment over a 6-month period react the same as naïve snakes). Data patterns suggested that snakes that did not experience harassment had a higher magnitude of CORT response than snakes who had been repeatedly harassed. It is possible that the snakes that experienced repeated harassment became habituated to the harassment and did not have a strong physiological reaction at the end of the study. To my knowledge, there are no studies investigating the habituation of reptiles to a repeated stressor, and more research on the patterns from this research is needed. It is also important to investigate the effects of long-term, repeated harassment on the baseline CORT concentrations in rattlesnakes. Studies have shown that repeated translocation does not impact baseline CORT concentrations (Holding et al. 2014a, Heiken et al. 2016). However, these studies did not include harassment as a stressor and did not last as long as the study conducted in Chapter 3 (6 months). We know that the harassment strategy used in this study successfully raises CORT in *C. atrox*. Further research is needed on the effects of repeated harassment on the physiology and behaviors of rattlesnakes.

The results from this study show that the harassment method raises plasma CORT concentrations to a “maximum” level above baseline CORT concentrations and elicits an acute stress response. Trompeter and Langkilde (2011) found that lizards (*Sceloporus undulatus*) were able to adapt to a novel predator by changing their natural anti-predator behaviors to avoid interactions with invasive ants. With the knowledge that reptiles can change their behaviors in response to a novel predator or stressor (see also Thaker et al. 2009; 2010), it could be possible for rattlesnakes to learn anti-predator behaviors to avoid humans or areas with high human activity. With previous knowledge of reptile learning and adaptations to novel predators and additional research on the patterns observed in Chapter 3, the results of this study could be used to inform research on management strategies to minimize human-rattlesnake interactions. For example, a larger sample size might allow for more evident patterns in a study examining the effectiveness of the harassment method coupled with SDT (Chapter 3). If effective, this two-pronged approach could be used in high-traffic public use areas (e.g., around dwellings, along trails) within areas such as national parks and monuments where other management strategies are not available. Pending additional research on its effectiveness, this method could also be used to deter rattlesnakes from human-developed areas such as housing communities and businesses. Managing human-rattlesnake interactions in these areas is critical to public and environmental health.

LITERATURE CITED

Amarello, M., E.M. Nowak, E.N. Taylor, G.W. Schuett, R.A. Repp, P.C. Rosen, and D.L. Hardy, Sr. 2010. Potential environmental influences on variation in body size and sexual dimorphism among Arizona populations of the western diamond-backed rattlesnake (*Crotalus atrox*). Journal of Arid Environments 74: 1443-1449.

Bailey, F.C., V.A. Cobb, T.R. Rainwater, T. Worrall, and M. Kluwowski. 2009. Adrenocortical effects of human encounters of free-ranging Cottonmouths (*Agkistrodon piscivorus*). Journal of Herpetology 43: 260-266.

Beylin, A.V., and T.J. Shors. 2003. Glucocorticoids are necessary for enhancing the acquisition of associative memories after acute stressful experience. Hormones and Behavior 43: 124-131.

Brown, J., C. Bishop, and R. Brooks. 2009. Effectiveness of short-distance translocation and its effects on western rattlesnakes. Journal of Wildlife Management 73: 419-425.

Brusch, G.A., and D.F. DeNardo. 2017. When less means more: dehydration improves innate immunity in rattlesnakes. Journal of Experimental Biology 220(12): 2287-2295.

Burchfield, S. R. 1979. The stress response: a new perspective. Psychosomatic Medicine 41: 661-672.

Butler, H., B. Malone, and N. Clemann. 2005. The effects of translocation on the spatial ecology of tiger snakes (*Notechis scutuatus*) in a suburban landscape. Wildlife Research 32: 165-171.

Campbell, J.A., and W.W. Lamar. 2004. The venomous reptiles of the Western Hemisphere, Volume 2. Cornell University Press(Comstock Publishing Associates), Ithaca, New York, USA.

Capehart, G.D., C. Escallon, B.J. Vernasco, I.T. Moore, and E.N. Taylor. 2016. No drought about it: effects of supplemental hydration on the ecology, behavior, and physiology of free-ranging rattlesnakes. Journal of Arid Environments 134: 79-86.

Claunch, N.M., J.A. Frazier, C. Escallon, B.J. Vernasco, I.T. Moore, and E.N. Taylor. 2017. Physiological and behavioral effects of exogenous corticosterone in a free-ranging ectotherm. General and Comparative Endocrinology 248: 87-96.

Corneille, M.G., S. Larson, R.M. Stewart, D. Dent, J.G. Myers, P.P. Lopez, M.J. McFarland, and S.M. Cohn. 2006. A large single-center experience with treatment patients with crotalid envenomations: outcomes with and evolution of antivenin therapy. American Journal of Surgery 192: 848-852.

Dart, R. C., J.T. McNally, D. W. Spaite, and R. Gustafson. 1992. The sequelae of pitviper poisoning in the United States. Pp. 395-404 *In* J.A. Campbell and E.D. Brodie, Jr. (Eds.), Biology of the Pitvipers, Selva, Tyler, Texas.

Dayger, C.A., and D. I. Lutterschmidt. 2016. Seasonal and sex differences in responsiveness to adrenocorticotropic hormone contribute to stress response plasticity in red-sided garter snakes (*Thamnophis sirtalis parietalis*). Journal of Experimental Biology 219: 1022-1030.

Diamandis, E.P., and T.K. Christopoulos. 1996. Immunoassay. Academic Press, San Diego.

Dickens, M.J., D.J. Delehanty, and L.M. Romero. 2010. Stress: an inevitable component of animal translocation. Biological Conservation 143: 1329-1341.

Dillon, D., A.F. Ajo, K.E. Hunt, and C.L. Buck. 2021. Investigation of keratinase digestion to improve steroid hormone extraction from diverse keratinous tissues. General and Comparative Endocrinology 309: 113795.

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

Engel, T.R. 1997. Seed dispersal and plant regeneration by snakes? Ecotropica 3: 33-41.

Fernández Ajó, A.A., K.E. Hunt, M. Uhart, V. Rowntree, M. Sironi, C.F. Marón, M.D. Martino, and C.L. Buck. 2018. Lifetime glucocorticoid profiles in baleen of right whale calves: potential relationships to chronic stress of repeated wounding by Kelp Gulls. Conservation Physiology 6: 1-12.

Found, R., and C.C. St. Claire. 2018. Personality influences wildlife responses to aversive conditioning. The Journal of Wildlife Management 82: 747-755.

Found, R., E.L. Kloppers, T.E. Hurd, and C.C. St. Claire. 2018. Intermediate frequency of aversive conditioning best restores wariness in habituated elk (*Cercus canadensis*). PLoS One 13: e0199216. https://doi.org/10.1371/journal.pone.0199216.

French, S.S., H.B. Fokidis, and M.C. Moore. 2008. Variation in stress and innate immunity in the Tree Lizard (*Urosaurus ornatus*) across an urban-rural gradient. Journal of Comparative Physiology 178: 997-1005.

Frigerio, D., J. Dittami, E. Möstl, and K. Kotrschal. 2004. Excreted corticosterone metabolites co-vary with ambient temperature and air pressure in male Greylag geese (*Anser anser*). General and Comparative Endocrinology 137: 29-36.

Greenberg, N. 2002. Ethological aspects of stress in a model lizard, *Anolis carolinensis*. Integrative and Comparative Biology 42: 526-540.

Halsey, L.G. 2019. The reign of the p-value is over: what alternative analyses could we employ to fill the power vacuum? Biology Letters 15: 20190174.

Hardy, D.L., Sr., H.W. Greene, B. Tomberlin, and M. Webster. 2001. Relocation of nuisance rattlesnakes: problems using short-distance translocation in a small rural community. Pp. 44-45 *In* W.L. Halvorson and B.S. Gebow (Eds.), Creative Cooperation in Resource

Management: Third Conference on Research and Resource Management in the Southwest Desert, Extended Abstracts. USGS Sonoran Desert Field Station, University of Arizona, Tucson, Arizona.

Hawley, J.E., T.M. Gehring, R.N. Schultz, S.T. Rossler, and A.P. Wydeven. 2009. Assessment of shock collars as nonlethal management for wolves in Wisconsin. Journal of Wildlife Management 73: 518-525.

Heiken, K.H., G.A. Brusch, S. Gartland, C. Escallon, I.T. Moore, and E.N. Taylor. 2016. Effects of long distance translocation on corticosterone and testosterone levels in male rattlesnakes. 2016. General and Comparative Endocrinology 237: 27-33.

Herr, M.W., S.P. Graham, and T. Langkilde. 2017. Stressed snakes strike first: Hormone levels and defensive behavior in free ranging cottonmouths (*Agkistrodon piscivorus*). General and Comparative Endocrinology 243: 89-95.

Holding, M.L., J.A. Frazier, S.W. Dorr, S.N. Henningsen, I.T. Moore, and E.N. Taylor. 2014a. Physiological and behavioral effects of repeated handling and short-distance translocation on free-ranging Northern Pacific Rattlesnakes (*Crotalus oreganus oreganus*). Journal of Herpetology 48: 233-239.

Hunt, K., N. Lysiak, M. Moore, R. Seton, L. Torres, and C. Buck. 2017b. Multiple steroid and thyroid hormones detected in baleen from eight whale species. Conservation Physiology 5: cox061.

Hunt, K.E., N.SJ. Lysiak, C.JD. Matthews, C. Lowe, A. Fernández Ajó, D. Dillon, C. Willing, M.P. Heidi-Jørgensen, S.H. Ferguson, M.J. Moore, and C.L. Buck. 2018. Multi-year patterns in testosterone, cortisol and corticosterone in baleen from adult males of three whale species. Conservation Physiology 6: coy049.

Jessop, T.S., M. Hamann, M.A. Read, and C.J. Limpus. 2000. Evidence for a hormonal tactic maximizing green turtle reproduction in response to a pervasive ecological stressor. General and Comparative Endocrinology 118: 407-417.

Klauber, L.M. 1972. Rattlesnakes: Their Habits, Life Histories, and Influence on Mankind, 2 Vols., 2nd ed. University of California Press, Berkeley and Los Angeles.

Kloppers, E.L., C. C. St. Clair, and T.E. Hurd. 2005. Predator-resembling aversive conditioning for managing habituated wildlife. Ecology and Society 10(1): 31.

Klukowski, M. 2011. Effects of breeding season, testosterone, and ACTH on the corticosterone response of free-ranging male fence lizards (*Sceloporus undulatus*). General and Comparative Endocrinology 173(2): 295-302.

Krochmal, A.R., A.J. Place, T.J. LaDuc, and T.C. Roth II. 2018. Phylogenetic patterns in learning and decision making in pit vipers (Viperidae: Crotalinae). Animal Behaviour 145: 117-123.

Lance, V.A. 1990. Stress in reptiles. *In* A. Epple, C.G. Scanes, and M.H. Stetson (eds.), Progress in Comparative Endocrinology, pp. 461-466. Wiley-Liss, New York.

Lance, V.A., J.S. Grumbles, and D.C. Rostal. 2001. Sex differences in plasma corticosterone in desert tortoises, *Gopherus agassizii*, during the reproductive cycle. Journal of Experimental Zoology 289: 285-289.

Langkilde, T. and R. Shine. 2006. How much stress do researchers inflict on their study animals? A case study using a scincid lizard, *Eulamprus heatwolei*. Journal of Experimental Biology 209: 1035-1043.

Lauger, K.K. 2019. Stress Physiology, Behavior, and Growth of Federally Threatened Narrow-headed Gartersnakes in Captivity. Unpublished Master's Thesis, Northern Arizona University, Flagstaff, Arizona, USA.

Lentini, A.M. 2008. Investigating behavioural and physiological effects of surgically implanted transmitters on Massasauga rattlesnakes. Unpublished Doctoral dissertation, York University, Toronto, Ontario, Canada.

Mahmoud, I.Y, K. Vliet, L.J. Guillette Jr., and J.L. Plude. 1996. Effect of stress and $\mathrm{ACTH}_{1\text{-}24}$ on hormonal levels in male alligators, *Alligator mississippiensis*. Comparative Biochemistry and Physiology Part A: Physiology 115(1): 57-62.

Mathies, T., T.A. Felix, and V.A. Lance. 2001. Effects of trapping and subsequent short-term confinement stress on plasma corticosterone in the brown treesnake (*Boiga irregularis*) on Guam. General and Comparative Endocrinology 124: 106-114.

Mazur, R.L. 2010. Does aversive conditioning reduce human-black bear conflict? Journal of Wildlife Management 74: 48-54.

McDiamrmid, R.W., M.S. Foster, C. Guyer, J.W. Gibbons, and N. Chernoff. 2012. Reptile Biodiversity: Standard Methods for Inventory and Monitoring. University of California Press, Berkley and Los Angeles, California.

Minckley, W.L. and D.E. Brown. 1994. Tropical-subtropical wetlands. Pp. 268-287 *In* D.E. Brown (Ed.), Biotic Communities of the Southwestern United States and Mexico. University of Utah Press, Salt Lake City.

Moeller, K.T., G. Demare, S. Davies, and D.F. DeNardo. 2017. Dehydration enhances multiple physiological defense mechanisms in a desert lizard, *Heloderma suspectum*. Journal of Experimental Biology 220: 2166-2174.

Moore, I. T., J.P. Lerner, D.T. Lerner, R.T. Mason. 2000. Relationships between annual cycles of testosterone, corticosterone, and body condition in male red-spotted garter snakes, *Thamnophis sirtalis concinnu.* Physiological and Biochemical Zoology 73: 307-312.

Moore, I.T. and T.S. Jessop. 2003. Stress, reproduction, and adrenocortical modulation in amphibians and reptiles. Hormones and Behavior 43: 39-17.

Moynihan, J.A. 2003. Mechanisms of stress-induced modulation of immunity. Brain, Behavior, and Immunity 17: S11-S16.

National Park Service. 1980. United States Codes: Department of the Interior: National Park Service Organic Act. Title 16, Subchapter 1.

National Park Service. 2022. Annual visitation by park. https://irma.nps.gov/Stats/SSRSReports/Park%20Specific%20Reports/Annual%20Park%20Recreation%20Visitation%20(1904%20-%20Last%20Calendar%20Year)?Park=MOCA. Accessed 4, January 2023.

National Park Service. 2022. Annual visitation by park. https://irma.nps.gov/Stats/SSRSReports/Park%20Specific%20Reports/Annual%20Park%20Recreation%20Visitation%20(1904%20-%20Last%20Calendar%20Year)?Park=TUZI; Accessed 4, January 2023.

Nowak, E.M. 2005. Movement patterns and life history of wester diamond-backed rattlesnakes (*Crotalus atrox*) at Tuzigoot National Monument, Arizona. Pp. 235-274 *In* C. van Riper III and D. Mattson, (Eds.), The Colorado Plateau II: Biophysical, socioeconomic, and cultural research. University of Arizona Press, Tucson, Arizona.

Nowak, E.M. and C. van Riper III. 1999. Effects and effectiveness of rattlesnake relocation at Montezuma Castle National Monument. U.S. Geological Survey/FRESC Report Series USGS/FRESC/COPL/1999/17.

Nowak, E.M. and H.W. Greene. 2016. Rattlesnake Conservation in the 21st Century. Pp 413-458 *In* G.W. Schuett, M.J. Feldner, C.F. Smith, and R.S. Reiserer (Eds.), Rattlesnakes of Arizona, Volume 2: Conservation, Behavior, Venom, and Evolution. ECO Herpetological Publishing, Rodeo, New Mexico.

Nowak, E.M., T.C. Theimer, and G.W. Schuett. 2008. Functional and numerical responses of predators: Where do vipers fit in the traditional paradigms? Biological Review 83: 601-620.

Peterson, C.E., and J.B. Sealy. 2021. Threats and Conservation Status. Pp. 81-98 *In* Breisch, A.R., W.H. Martin, J.B. Sealy, C.E. Peterson, and E. Possardt (Eds). The Timber Rattlesnake: Life History, Distribution, Status, and Conservation Action Plan. Partners in Amphibian and Reptile Conservation Technical Publication CAP-1. Published by the Amphibian and Reptile Conservancy, Nashville, Tennessee.

Rauer, G., P. Kaczensky, and F. Knauer. 2003. Experiences with aversive conditioning of habituated brown bears in Austria and other European countries. Ursus 14: 215-224.

Reinert, H.K., and R.R. Rupert. 1999. Impacts of translocation on behavior and survival of timber rattlesnakes, *Crotalus horridus*. Journal of Herpetology 33: 45-61.

Reiserer, R.S., G.W. Schuett, and H.W. Greene. 2018. Seed ingestion and germination in rattlesnakes: overlooked agents of rescue and secondary dispersal. Proceedings of the Royal Society B: Biological Sciences 285: 20172755.

Romero, L., and M. Wikelski. 2001. Corticosterone levels predict survival probabilities of Galapagos marine iguanas during El Nino events. Proceedings of the National Academy of Sciences of the United States of America 13: 7366-7370.

Romero, L.M. 2004. Physiological stress in ecology: lessons from biomedical research. Trends in Ecology and Evolution 19: 249-255.

Romero, L.M., and J.C. Wingfield. 1998. Seasonal changes in adrenal sensitivity alter corticosterone levels in Gambel's White-Crowned Sparrows (*Zonotrichia leucophrys gambelii*). Comparative Biochemistry and Physiology 199C: 31-36.

Sapolsky, R.M., L.M. Romero, and A.U. Munck. 2000. How do glucocoirticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocrine Reviews 21: 55-89.

Schuett, G.W., E.N. Taylor, E.A. Van Kirk, and W.J. Murdoch. 2004. Handling stress and plasma corticosterone levels in captive male Western Diamond-backed Rattlesnakes (*Crotalus atrox*). Herpetological Review 35:229-233.

Sealy, J.B. 2002. Ecology and behavior of the Timber Rattlesnake (*Crotalus horridus*) in the upper piedmont of North Carolina: Identified threats and conservation recommendations. Biology of the Vipers 2002: 561-578.

Silvestre, A.M. 2014. How to assess stress in reptiles. Journal of Exotic Pet Medicine 23: 240-243.

Sonderegger, D.L. 2020. Statistical Methods II. Bookdown.org. https://bookdown.org/dereksonderegger/571/.

Sonderegger, D.L., and R. Buscaglia. 2020. Introduction to Statistical Methodology, Second Edition. Bookdown.org. https://bookdown.org/dereksonderegger/570/.

Sullivan, B.K., E.M. Nowak, and M.A. Kwiatkowski. 2014. Mitigation translocation of nuisance herpetofauna: can it be improved? Conservation Biology 29: 12-18.

Sun, L., R. Shine, Z. Debi, T. Zhengren. 2001. Biotic and abiotic influences on activity patterns of insular pit-vipers (*Gloydius shedaoensis*, Viperidae) from north-eastern China. Biological Conservation 97: 387-398.

Taylor, E.N, D.F. DeNardo, and D.H. Jennings. 2004. Seasonal steroid hormone levels and their relation to reproduction in the Western Diamond-backed Rattlesnake, *Crotalus atrox* (Serpentes: Viperidae). General and Comparative Endocrinology 136(3): 328-337.

Taylor, E.N. and W. Booth. 2016. Rattlesnakes as Models for Reproductive Studies of Vertebrates. Pp 121-157 *In* G.W. Schuett, M.J. Feldner, C.F. Smith, and R.S. Reiserer

(Eds.), Rattlesnakes of Arizona, Volume 2: Conservation, Behavior, Venom, and Evolution. ECO Herpetological Publishing, Rodeo, New Mexico.

Thaker, M., A.T. Vanak, S.L. Lima, and D.K. Hews. 2010. Stress and aversive learning in a wild vertebrate: the role of corticosterone in mediating escape from a novel stressor. The American Naturalist 175: 50-60.

Thaker, M., S. Lima, and D.K. Hews. 2009. Acute corticosterone elevation enhances antipredator behaviors in male tree lizard morphs. Hormones and Behavior 56: 51-57.

Tokish, J.T., J. Benjamin, and F. Walter. 2001. Crotalid envenomation: the southern Arizona experience. Journal of Orthopaedic Trauma 15: 5-9.

Trevisi, E. and G. Bertoni. 2009. Some physiological and biochemical methods for acute and chronic stress evaluation in dairy cows. Italian Journal of Animal Science 8: 265-286.

Trompeter, W.P., and T. Langkilde. 2011. Invader danger: lizards faced with novel predators exhibit an altered behavioral response to stress. Hormones and Behavior 60: 152-158

Turner, R.M. and D.E. Brown. 1994. Tropical-subtropical desertland. Pp. 180-221 *In* D.E. Brown (Ed.), Biotic Communities of the Southwestern United States and Mexico. University of Utah Press, Salt Lake City.

Wingfield, J.C. and L.M. Romero. 2001. Adrenocortical responses to stress and their modulation in free-living vertebrates. *In* B.S. McEwen and H.M. Goodman (eds.), Handbook of Physiology: The Endocrine System, Vol IV, Section 7, pp. 211-234. Oxford University Press, New York.

Wolfe, A.K., P.A. Fleming, and P.W. Bateman. 2018. Impacts of translocation on a large urban-adapted venomous snake. Wildlife Research 45: 316-324.

Young, K.M., S.L. Walker, C. Lanthier, W.T. Waddell, S.L. Monfort, J.L. Brown. 2004. Noninvasive monitoring of adrenocortical activity in carnivores by fecal glucocorticoid analyses. General and Comparative Endocrinology 137: 148-165.

TABLE CAPTIONS

TABLE 2-1. Western Diamond-backed Rattlesnakes (*Crotalus atrox*) captured for this study at Montezuma Castle National Monument, Tuzigoot National Monument, and the Apache Junction site between August 2021 and September 2022. The snakes were bled to assess plasma corticosterone levels at baseline, post-harassment, and post-adrenocorticotropin treatment. The table includes the date captured, field site, sex, snout to vent length (SVL) in millimeters (mm), and mass in grams (g) for each snake. Each "X" represents data that was lost for 17 snakes.

Table 2-2. F-test (parallelism) and linear regression (accuracy) results of CORT EIA biochemical validation tests for Western Diamond-backed Rattlesnakes (*Crotalus atrox*) plasma samples. The parallelism test compared the slopes of the linear portion of the binding curves of assay standards verses serial diluted samples. The accuracy test shows the slope of the observed vs expected doses (slope should be between 0.7 and 1.3) and a correlation coefficient (R^2 should be close to 1) of the linear regression line.

TABLE 2-3. Linear mixed effects models examining effects of sex and treatment group on plasma corticosterone levels at baseline, post-harassment, and post-adrenocorticotropin treatment on wild adult Western Diamond-backed Rattlesnakes (*Crotalus atrox*) from three sites in Arizona during 2021-2022. Snake ID was included as a random effect to account for multiple samples taken from the same snake. Model number and model variables, Akaike Information Criteria (AIC), models included in Analysis of Variance (ANOVA), and resulting p-values for each test are given. Pairwise comparisons of model variables using Tukey's Post-hoc tests are also given. Data included the outlier snake "Stinky", who had a post-adrenocorticotropin corticosterone level that was lower than her baseline corticosterone level.

FIGURE LEGENDS

FIGURE 2-1. Accuracy validation for plasma corticosterone in wild adult Western Diamond-backed Rattlesnakes (*Crotalus atrox*) sampled from field sites in Apache Junction, Pinal County, Arizona, and Tuzigoot and Montezuma Castle National Monument, Yavapai County, Arizona from August 2021 to September 2022. The snakes were bled to assess corticosterone levels at baseline, post-harassment, and post-adrenocorticotropin treatment.

FIGURE 2-2. Parallelism validation for plasma corticosterone in wild adult Western Diamond-backed Rattlesnakes (*Crotalus atrox*) sampled from field sites in Pinal County and Yavapai County, Arizona from August 2021 to September 2022. The snakes were bled to assess corticosterone levels at baseline, post-harassment, and post-adrenocorticotropin treatment.

FIGURE 2-3. Plasma corticosterone levels of wild adult Western Diamond-backed Rattlesnakes (*Crotalus atrox*) sampled in Apache Junction, Pinal County, Arizona, and Tuzigoot and Montezuma Castle National Monument, Yavapai County, Arizona from August 2021 to September 2022. The snakes were bled to assess corticosterone levels at baseline, post-harassment, and post-adrenocorticotropin treatment. Treatment groups are: "A" (adrenocorticotropin treated snakes), "B" (baseline group), and "H" (harassed group). Data included the outlier snake "Stinky" that had a post-adrenocorticotropin corticosterone level that was lower than her baseline corticosterone level.

FIGURE 2-4. Plasma corticosterone levels of "F" (female) and "M" (male) wild adult Western Diamond-backed Rattlesnakes (*Crotalus atrox*) sampled in Apache Junction, Pinal County, Arizona, and Tuzigoot and Montezuma Castle National Monument, Yavapai County, Arizona from August 2021 to September 2022. The snakes were bled to assess plasma corticosterone levels at baseline, post-harassment, and post-adrenocorticotropin treatment. Data included the outlier snake "Stinky" that had a post-adrenocorticotropin corticosterone level that was lower than her baseline corticosterone level.

FIGURE 2-5. Interaction plot to assess the interaction between group ("A" (post-ACTH), "B" (baseline), "H" (harassed)) and sex ("F" (females), "M" (male)) in plasma corticosterone levels of wild adult Western Diamond-backed Rattlesnakes (*Crotalus atrox*) sampled in Apache Junction, Pinal County, Arizona, and Tuzigoot and Montezuma Castle National Monument, Yavapai County, Arizona from August 2021 to September 2022. The snakes were bled to assess corticosterone levels at baseline, post-harassment, and post-adrenocorticotropin treatment. Data included the outlier snake "Stinky" that had a post-adrenocorticotropin corticosterone level that was lower than her baseline corticosterone level.

FIGURE 2-6. Mean plasma corticosterone levels of wild adult Western Diamond-backed Rattlesnakes (*Crotalus atrox*) sampled in Apache Junction, Pinal County, Arizona, and Tuzigoot and Montezuma National Monument, Yavapai County, Arizona from August 2021 to September 2022. The snakes were bled to assess corticosterone levels at baseline, post-harassment, and post-adrenocorticotropin treatment. Corticosterone levels are separated by sex "F" (female; orange) and "M" (male; blue) and by groups, "B" (baseline), "H" (post-harassment), and "A" (post-ACTH). Data included the outlier snake "Stinky" that had a post-adrenocorticotropin corticosterone level that was lower than her baseline corticosterone level.

FIGURE 2-7. Plasma corticosterone levels of wild adult Western Diamond-backed Rattlesnakes (*Crotalus atrox*) sampled in Apache Junction, Pinal County, Arizona, and Tuzigoot and Montezuma Castle National Monument, Yavapai County, Arizona from August 2021 to September 2022. The snakes were bled to assess corticosterone levels at baseline, post-harassment, and post-adrenocorticotropin treatment. Corticosterone levels taken from the same snake identified by “Snake_Name” include Group “A” (post-ACTH; orange) and Group “B” (baseline; blue). Data included the outlier snake “Stinky” that had a post-adrenocorticotropin corticosterone level that was lower than her baseline corticosterone level.

TABLE 2-1

Date Captured	Field Site	Sex	Number of Individuals	SVL (mm)	Mass (g)
8/17/21	Apache Junction	F	4	X	X
8/17/21	Apache Junction	M	1	X	X
8/27/21	Apache Junction	M	1	X	X
8/27/21	Apache Junction	F	1	X	X
8/28/21	Apache Junction	M	3	X	X
9/1/21	MOCA	M	1	X	X
9/5/21	Apache Junction	M	1	X	X
9/5/21	Apache Junction	F	1	X	X
9/10/21	MOCA	M	1	X	X
9/11/21	Apache Junction	F	1	X	X
9/12/21	MOCA	F	2	X	X
9/18/21	Apache Junction	F	1	765	305
9/18/21	Apache Junction	F	1	700	250
9/19/21	Apache Junction	M	1	845	369
9/19/21	Apache Junction	M	1	808	324
9/25/21	Apache Junction	F	1	831	342
9/8/22	MOCA	M	1	817	485
9/19/22	MOCA	F	1	887	459
9/26/22	TUZI	M	1	990	871

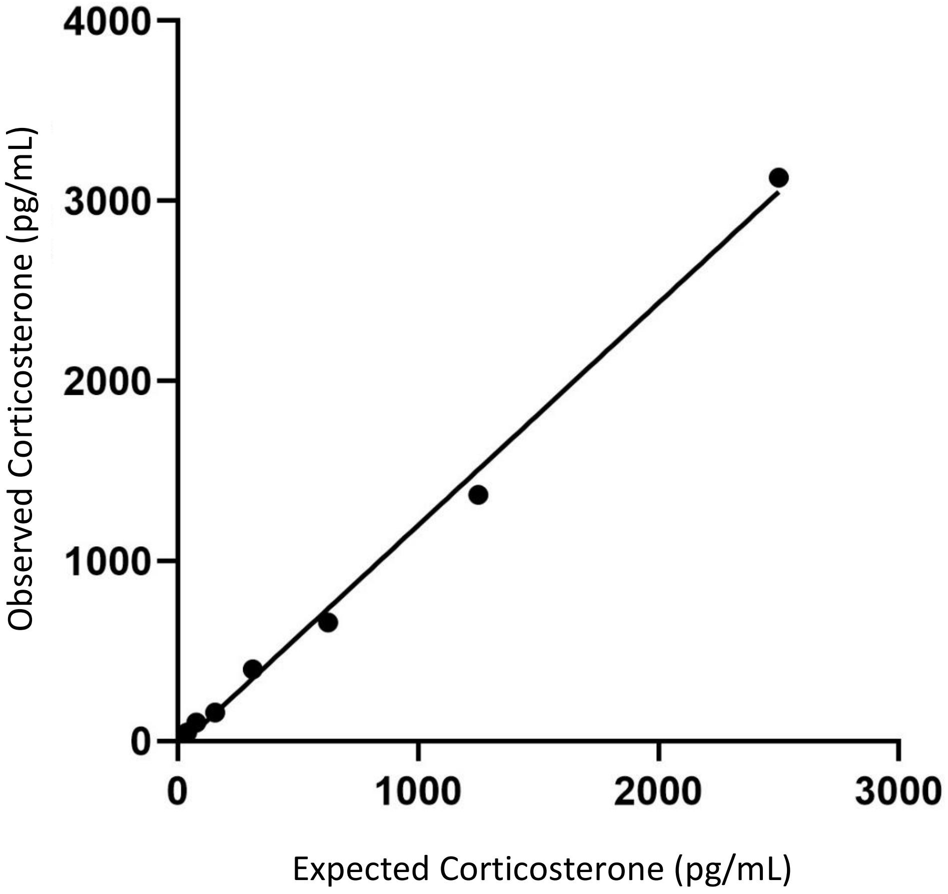
TABLE 2-2.

Species	**Parallelism**	**Accuracy**
Crotalus atrox	$F_{1,11}$= 0.1348 p= 0.7205	Slope= 1.235 R^2=0.9950

TABLE 2-3.

Model #	Model	AIC	ANOVA	p-value	Pairwise comparisons	p-value
M1	ng.mL~Group+(1|Snake_ID)	21.389	M1 vs M2	0.0002		
M2	ng.mL~(1|Snake_ID)	34.145	M4 vs M1	0.4000		
M3	ng.mL~Group+(1|Snake_ID)+Sex+(Group:Sex)	25.775	M3 vs M4	0.6360		
M4	ng.mL~Group+(1|Snake_ID)+Sex	22.680	M4 vs M5	0.0002	ACTH-Baseline	0.0017
					ACTH-Harassed	0.7723
					Baseline-Harassed	0.0017
M5	ng.mL~Sex+(1|Snake_ID)	36.05				

FIGURE 2-1.



4000
3000
2000
1000
0
0
1000
2000
3000
Observed Corticosterone (pg/mL)
Expected Corticosterone (pg/mL)

FIGURE 2-2.

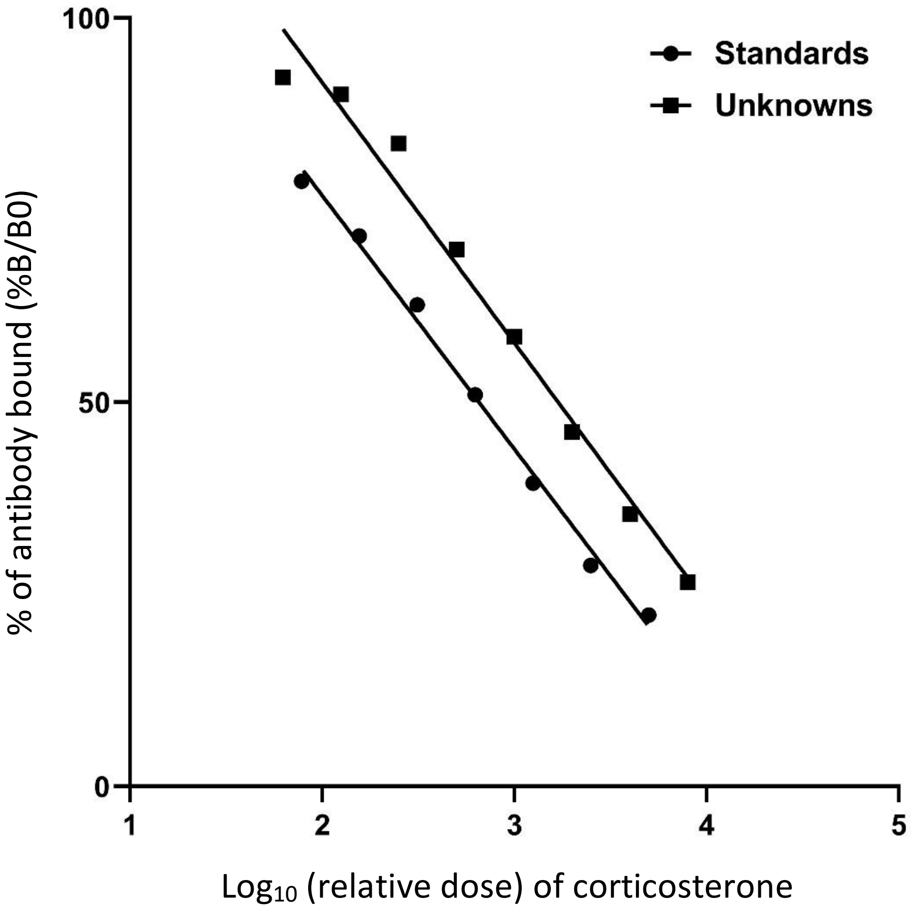

100
50
0
1
2
3
4
5
% of antibody bound (%B/B0)
Log10 (relative dose) of corticosterone
Standards
Unknowns

FIGURE 2-3

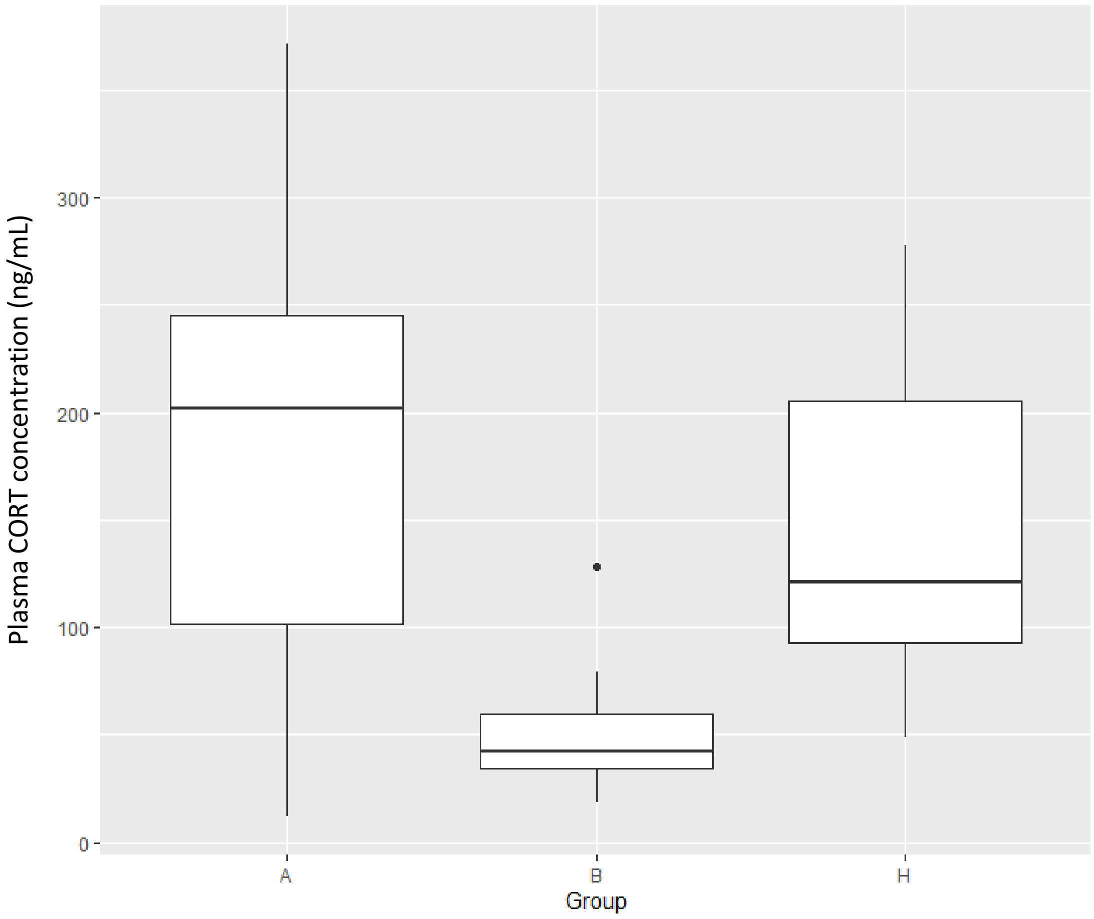


FIGURE 2-4

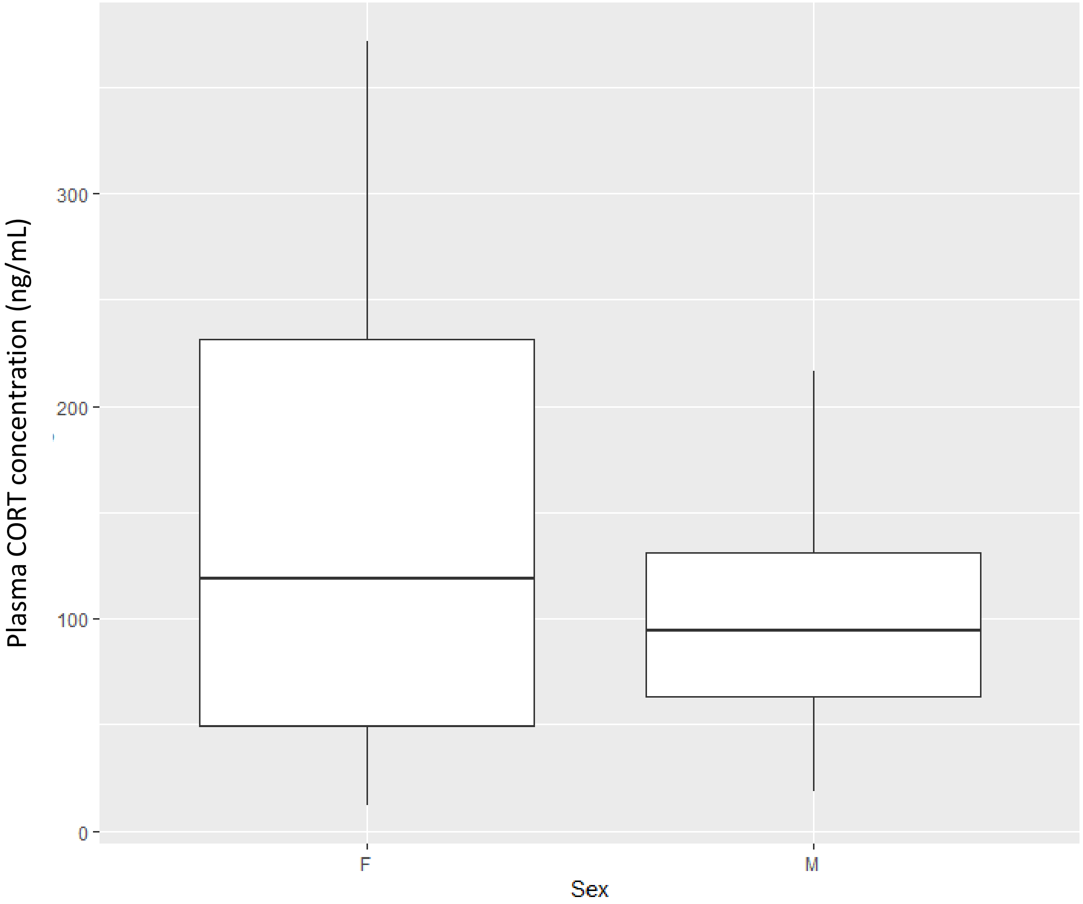


FIGURE 2-5

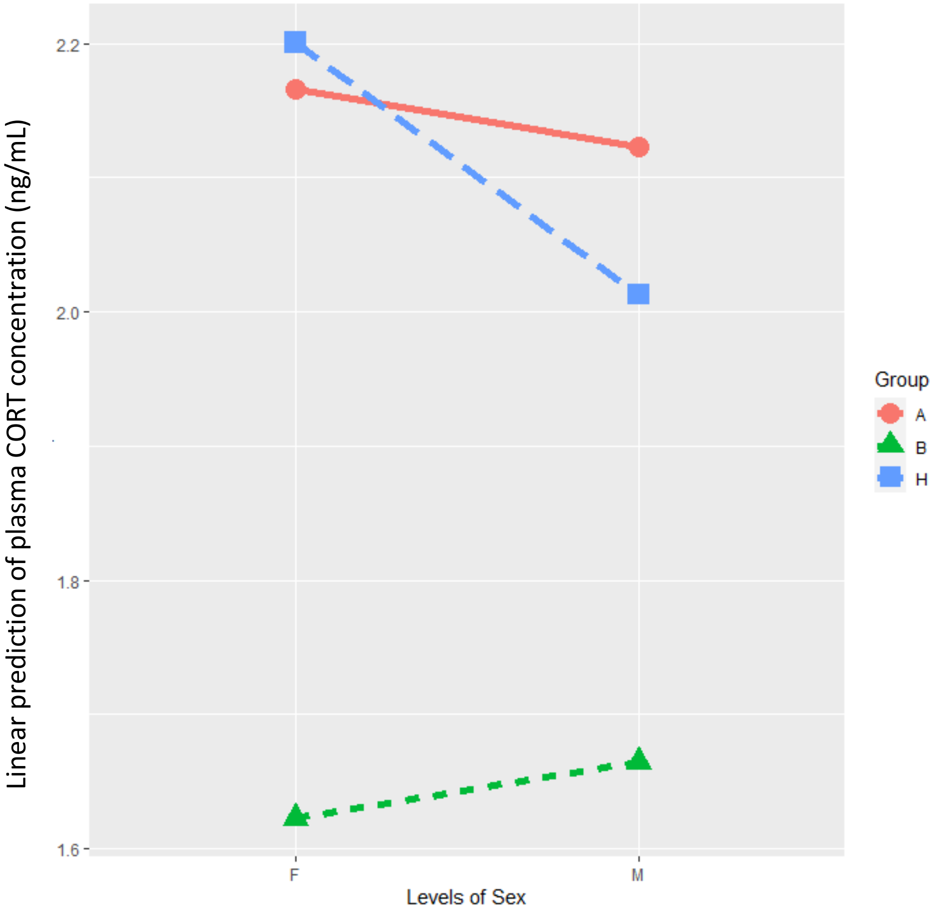

Linear prediction of plasma CORT concentration (ng/mL)
2.2
2.0
1.8
1.6
F
M
Levels of Sex
Group
A
B
H

FIGURE 2-6

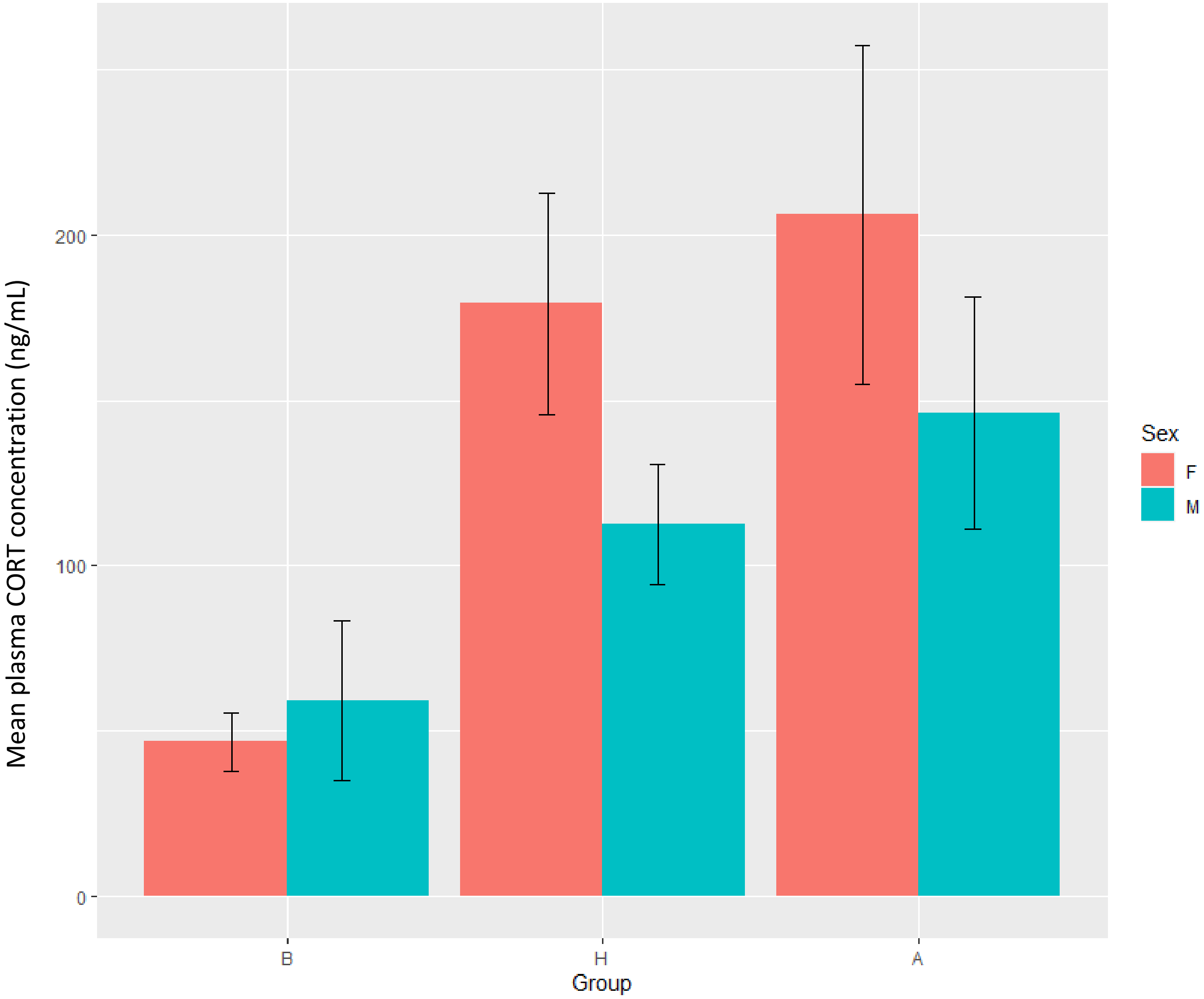
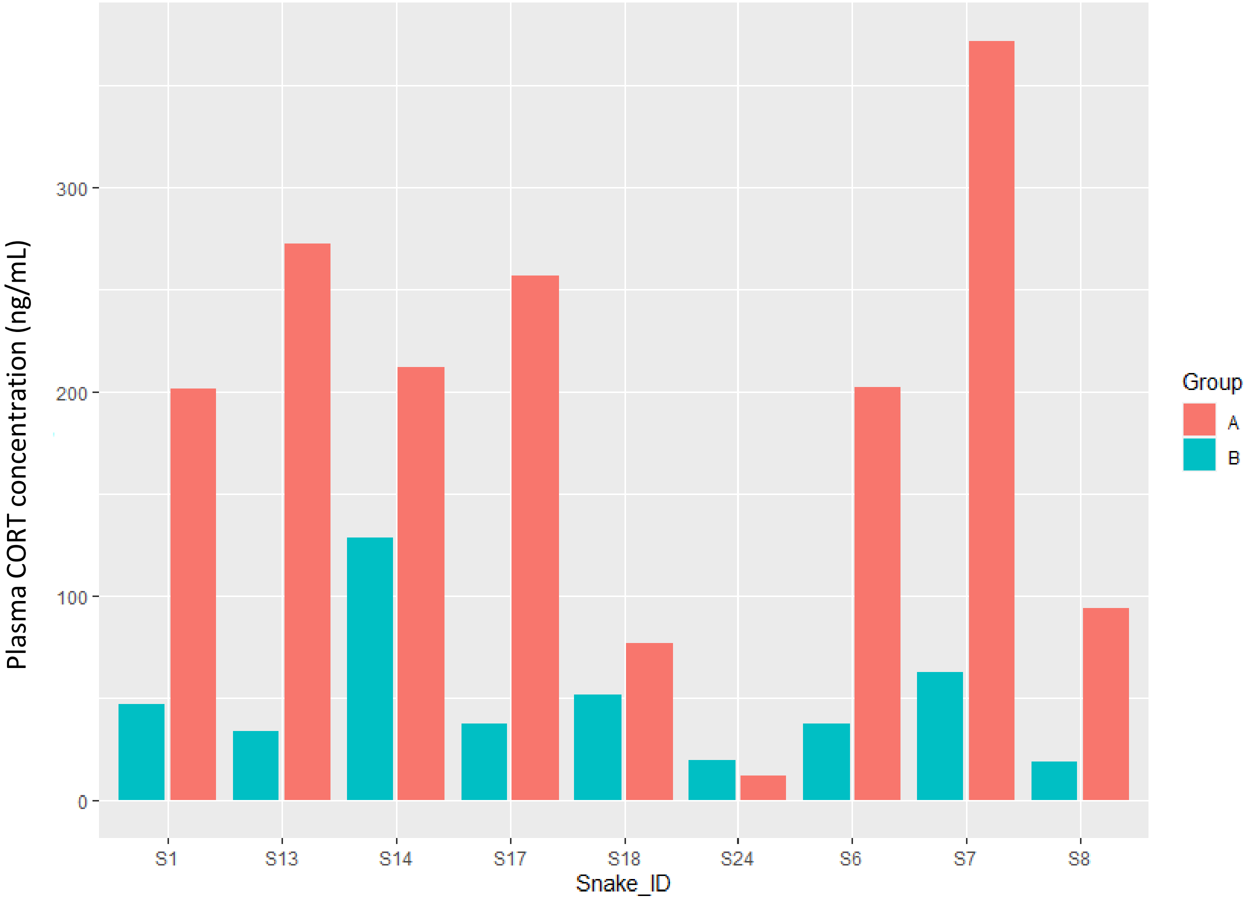

Mean plasma CORT concentration (ng/mL)
0
100
200
B
H
A
Group
Sex
F
M

FIGURE 2-7


Plasma CORT concentration (ng/mL)
300
200
100
0
S1
S13
S14
S17
S18
S24
S6
S7
S8
Snake_ID
Group
A
B

CHAPTER 3

BEHAVIORAL AND SPATIAL ECOLOGY CHANGES IN RESPONSE TO REPEATED HARASSMENT IN WESTERN DIAMOND-BACKED RATTLESNAKES

Introduction

Rattlesnakes contribute greatly to the ecosystems in which they inhabit (Klauber 1972, Engel 1997, Sun et al. 2001, Nowak et al. 2008, Reiserer et al. 2018). These contributions, including serving as predator, prey, and seed dispersal and germination, provide justification for protecting rattlesnakes, especially in areas where they come into contact with humans and the snakes face persecution (Greene and Campbell 1992; Greene 1997, 2013; Nowak and Greene 2016). Sullivan et al. (2017) showed that Western Diamond-backed Rattlesnakes (*Crotalus atrox*) were one of the most common snakes in urban environments in a desert community. Due to an increased risk of venomous snakebite (Dart et al. 1992, Tokish et al. 2001, Campbell and Lamar 2004, Corneille et al. 2006) as well as high risk of snake mortality from human predators (Reinert and Rupert 1999, Sullivan et al. 2014, Nowak and Greene 2016), rattlesnakes in urban environments are often removed. Currently, the most common method of removing snakes from urban environments is translocation, yet this method may be ineffective (Sullivan et al. 2014) and is often detrimental to snake health and survival (Reinert and Rupert 1999, Nowak et al. 2002, Brown et al. 2011).

One method that can be used to address human-wildlife conflicts is the use of behavior-based methods such as aversive conditioning, which attempts to replace neutral or positive associations with human activity with negative associations through fear or discomfort (Found and St. Clair 2018). This method has been successful in changing behaviors in nuisance bears (Mazur 2010, Rauer et al. 2003), elk (Kloppers et al. 2005, Found et al. 2018, Found and St.

Clair 2018), and wolves (Hawley et al. 2009). Found et al. (2018) separated elk into two groups experiencing a high frequency of aversive conditioning (i.e., 3-5 events over a 90-day period) and a low frequency (i.e., 6-9 events over a 90-day period); they found that the elk learned avoidance best at intermediate frequencies (i.e., frequencies that were not too high or too low).

Like all vertebrates, reptiles have the ability to learn and make decisions when faced with a negative stimulus. Multiple studies show that reptiles can modify their behavior in response to a negative stimulus such as a novel stressor or predator (Thaker et al. 2010, Trompeter and Langkilde 2011, Krochmal et al. 2018) and flavor aversion (Burghardt et al. 1973, Paradis and Cabanac 2004, Ward-Fear et al. 2017). Learning in rattlesnakes was demonstrated by Krochmal et al. (2018), who conducted a study demonstrating that rattlesnakes were able to decrease their latency in decision making in a thermal maze after only one trial, while non-rattlesnake pitviper relatives showed no modification in latency in decision making after twelve trials through the thermal maze. Lizards have also exhibited an ability to learn and change their behavior in response to novel predators (Trompeter and Langkilde 2011). Although there is compelling evidence that reptiles are able to adapt and learn in response to a negative stimulus such as flavor aversion (Burghardt et al. 1973, Paradis and Cabanac 2004, Ward-Fear et al. 2017), novel predators (Trompeter and Langkilde 2011), and heat (Krochmal et al. 2018), to my knowledge, the effects of long-term repeated harassment on wild rattlesnakes has not been studied.

Most instances of human-rattlesnake interactions result in rattlesnake relocation to maintain human and snake safety. Short-distance translocation (SDT) within the home range is likely a widely used management method to relocate nuisance rattlesnakes (reviewed in Sullivan et al. 2014, Nowak and Greene 2016). It is considered safer in most cases for nuisance snakes because it does not affect snake health or survival in most cases (Sealy 2002, Brown et al. 2009, Holding et al. 2014a). Therefore, short-distance translocation remains an oft-used component of practical rattlesnake management (Peterson and Sealy 2021). The use of harassment coupled with short-distance translocation could prove to be more effective in reducing human-rattlesnake interactions than short-distance translocation alone due to an increased level of stress and conditional learning (Hardy et al. 2001, Brown et al. 2009, Thaker et al. 2010, Trompeter and Langkilde 2011, Found et al. 2018, R. Clark, pers. comm.).

This project aims to determine if harassing and translocating free ranging telemetered rattlesnakes will be successful in eliciting behavioral and spatial ecology changes (after Langkilde and Shine 2006, Moeller et al. 2017). Using short-distance translocation as a constant variable in the study allowed us to assess the behavioral differences between snakes that experience harassment and snakes that are not harassed, while still considering the effects of short-distance translocation on the snakes. I used a harassment technique of placing rattlesnakes in 5-gallon snake-safe buckets and moving, flipping, lifting, and prodding them with snake tongs for 10 of every 30 seconds during a 30-minute period, and then translocating them a short distance (100m) to determine impacts on their behavior and spatial ecology (Langkilde and Shine 2006, Moeller et al. 2017). Ideally, comparing the home ranges, activity (core) ranges, frequency of movements, flight distances, and defensive behaviors between the treatment (translocated and harassed) and control (translocated) groups would show the effects of this repeated harassment. Comparing the differences between the two groups could give insight to the changes and impacts that harassment has in addition to translocation.

To validate that the behavioral responses in this study were due to increased stress, I first conducted a study to determine the effects of the harassment method on plasma corticosterone (CORT) levels in wild adult *C. atrox* (see Chapter 2). That study found that the harassment method successfully increased CORT levels above baseline levels. The rise in CORT levels after harassment were similar to post-adrenocorticotropin (ACTH) manipulated CORT levels (i.e., the harassment method successfully increased plasma CORT to a “maximum” level (see Chapter 2)). Reptiles use the physiological responses of stress responses to modify their behaviors in their environments (Thaker et al. 2010, Trompeter and Langkilde 2011, Holding et al. 2014a). Repeated intermittent harassment coupled with physical displacement from the harassment point could successfully change rattlesnake behavioral and spatial responses. Changes in rattlesnake behavior and spatial ecology could be used to help inform management strategies that could be used in a practical setting to decrease human-rattlesnake interactions.

Methods

Study Site

Tuzigoot National Monument (TUZI) is located in Clarkdale, Yavapai County, Arizona at 1036 m elevation. The monument is located approximately 35 kilometers west of Montezuma Castle National Monument (MOCA; chapter 2) and is bordered on the west side by private property, bordered to the north by U.S. Forest Service land, and on the east and south by Arizona State Park land (including the Verde River Greenway). The Verde River runs outside and adjacent to the monument's borders. Vegetation surrounding the river is consistent with Sonoran Riparian Deciduous Forest Scrubland (Minckley and Brown 1994). Tavasci Marsh, a large freshwater marsh, is located in the monument with vegetation consistent with Sonoran Interior Marshland (Minckley and Brown 1994). Upland vegetation at the monument is consistent with the Creosote-bush-Crucifixionthorn Series of the Arizona Upland Subdivision of the Sonoran Desertscrub as well as vegetation often seen in the Mojave, Great Basin, and Chihuahuan deserts (Turner and Brown 1994). Tuzigoot averaged approximately 97,053 visitors per year between 2011 and 2021 (NPS.gov; accessed 4, January 2023) and is a long-term monitoring location for *C. atrox* (Nowak and van Riper III 1999, Nowak 2005, Amarello et al. 2010, Nowak and Greene 2016, Nowak unpubl. data).

Study Snakes

Five adult (SVL > 70cm, which is larger than the smallest sexually mature male found in *C. atrox* by Taylor and Booth (2016) male Western Diamond-backed Rattlesnakes (*Crotalus atrox*) were collected from TUZI. Only snakes that were greater than 550g were considered for the study to ensure that the implanted devices did not comprise of less than 5% of the snake's body mass. Snakes were captured using tongs during visual encounter surveys (McDiarmid et al. 2017) and during opportunistic encounters by trained park staff and volunteers (see Nowak and

Greene 2016) between March and April 2022. Upon capture, snakes were restrained in plastic tubes and snout-vent length (SVL), tail length (TL), and body mass were measured. Each snake was sexed and scanned with a PIT tag scanner (Biomark, Boise, Idaho) to determine if it had been previously captured and micro-chipped. If snakes were not already micro-chipped, a 10 mm passive integrated transponder (PIT) tag (Biomark, Boise, Idaho) was implanted. I transported the snakes in specially designed snake-safe buckets (designed by James Starkey, Cornville, Arizona) to Northern Arizona University's (NAU) animal surgical suite for surgery.

Surgical Procedures

When snakes arrived at NAU, they were held at NAU's Biological Science Annex in an animal housing room and kept in modified plastic containers with air holes punctured in container sides. Snakes were provisioned with ad libitum water, a snake heating mat placed beneath their containers, recycled paper bedding (CareFresh), and disposable cardboard shoe box shelters. Ambient light conditions in the space were set to local sunrise/sunset conditions. Snakes were held in these housing units until surgery could be scheduled, typically 1-4 days. On the day of surgery, the snakes were taken from their holding room and transferred to the surgical suite via snake-safe bucket. Once in the suite, they were restrained in a clear, plastic snake tube. The snakes were then anesthetized using isoflurane vapor and implanted with a radio-transmitter, an accelerometer (ACT), and implantable temperature datalogger, following the methods of Reinert (1992), Hardy and Greene (1999a, 1999b), Taylor et al. (2004), and DeSantis et al. (2020). Radiotransmitter devices (Holohil Systems, Ltd., Ontario, Canada; model SI-2T) were ~ 11 grams, accelerometers (ACTs) (Technosmart Europe srl., Rome, Italy; model AXY-5) were ~9 grams, and temperature dataloggers (Thermochron iButtons, Maxim, Dallas, Texas) were ~3 grams and together comprised less than 5% of the snake's body mass. Radio-transmitters and

ACTs were coated in PlastiDip and implanted in the coelomic cavity of each snake following the implant procedures of Reinert (1992) and Hardy and Greene (1999a, 1999b) modified by DeSantis et al. (2020). Temperature dataloggers were implanted intracoelomically by securing them to the body wall; they were set to record temperature every 30-minutes. During post-operative recovery, the snakes were held in the same animal housing room with the same conditions mentioned above. Snakes were monitored for four days (longer if deemed necessary by NAU vet) post-surgery to ensure initial wound closure and healing and given a second dose of antibiotics (10 mg/kg of Enrofloxacin 2.27%). Once the snakes were recovered, they were returned to the exact location where they were originally captured. The animals were tracked at least three days a week and allowed to recover for two weeks before experiments began, following Nowak (1998).

Radio-transmitters, iButtons, and ACTs were removed from the snakes in September 2022 following the procedures as above. This removal timeline was to ensure that snakes had time to heal before the onset of the hibernation period (E. Nowak, pers. comm.). After recovery, snakes were returned to the exact location where they were previously captured.

Experimental Design

Snakes were located using a TR-4 or TR-5 receiver and a RA-23K antenna (Telonics Telemetry Consultants, Mesa, Arizona) at least three days a week (often more) during a 6-month period from April 2022 to September 2022. Upon release post-surgery, snakes were randomly assigned to a control or treatment group. Snakes in the treatment group were harassed and translocated 100 meters (SDT) opportunistically, but no more than once a calendar week following Holding et al. (2014a). The harassment method included placing the snakes in a snake-safe bucket and moving, flipping, lifting, and prodding with snake tongs for 10 of every 30 seconds of the 30-minute period (Langkilde and Shine 2006, Moeller et al. 2017). After the harassment was inflicted, the snakes were translocated using SDT in a random direction from their capture point approximately 100 meters. Snakes in the control group were short distance translocated approximately 100 meters opportunistically but no more than once a week (Holding et al. 2014a) and experienced no harassment. Snakes were located 24-hours after they experienced harassment and translocation or translocation alone to assess post-treatment flight distances. Implanted accelerometers were recording at 1 Hz for a 3–4-month period while snakes were located and harassed (DeSantis et al. 2020). The data obtained by radio-telemetry included activity and home range sizes, meters moved by the snakes each day, and flight distance away from the harassment site during the first 24-hours post-treatment (in meters). The data from the accelerometers showed the daily average Overall Dynamic Body Acceleration (ODBA), and iButtons showed body temperature data for the snakes every 30 minutes.

Defensive behavior was recorded during every radio-telemetric location of the snakes. Initial behavior was recorded when the observer was approximately 1-2 meters from the snake for 15 seconds, following Herr et al. (2017). Defensive behaviors were assigned to four categories (Cocks et al. 2019): Category 1 (None) was assigned to snakes that show no defensive behavior; Category 2 (Alert) was assigned to snakes that exhibited behaviors such as tongue flicking, freezing, tightening, or head raising; Category 3 (Avoid) was assigned to snakes that showed behaviors such as fleeing, retreating, or coiling; and Category 4 (Defend) included snakes that were observed hissing, rattling, posing to strike, or striking. The habitat/location and activity of each snake was documented whenever defensive behavior was recorded.

Enzyme Immunoassay of Plasma

Before each snake returned to NAU to remove their devices, they were harassed using the above harassment protocol. Following procedures described in Chapter 2, blood was drawn from each snake immediately after harassment to observe the difference in plasma corticosterone (CORT) concentrations between snakes that experienced harassment versus snakes that did not experience harassment. To collect a blood sample, the snakes were enticed into clear restraining tubes, and a 0.5 mL blood sample was collected from the caudal vein. I used a sterile 1.0 mL syringe with a 25g X 5/8" needle pre-treated with liquid heparin sodium and aimed to keep the blood drawing process below three minutes so that the blood collection process does not itself alter plasma CORT (Romero and Reed 2005). Blood samples were stored in 1.5 mL microtubes at ambient temperatures during field work and transferred to ice or a refrigerator during transport and storage until they could be prepared and stored in a -20°C freezer in the lab. These samples were prepared and analyzed using the same techniques outlined in Chapter 2. The biochemical validations for accuracy (Figure 2-1) and parallelism (Figure 2-2) are also applicable to these samples.

Statistical Analyses

All statistical analyses were performed in R Studio (Posit Software, PBC, version 2022.12.0) and Microsoft Excel (Microsoft Excel for Microsoft 365 MSO, version 2304). Data was tested for normality using the Shapiro-Wilk test and tested for homogeneity of variance using the Bartlett's test (Sonderegger and Buscaglia 2020). Non-parametric statistical analyses were performed on any data that was not normal. The analysis of distance moved per day excluding treatment day by group was analyzed using a Wilcoxon test (Hollander et al. 2013). The comparison of post-treatment flight distances by group and physiological responses to harassment among treatment groups were analyzed using a t-test (Sonderegger and Buscaglia

2020). The distance for post-treatment flight distance did not include the 100m translocation that snakes experienced (i.e., 24-hour post-treatment flight distance calculations began at the UTMs that the snake was moved to after translocation occurred). Any data that was not tested statistically was reported in tables with means and standard errors.

Accelerometer data was analyzed using Microsoft Excel (Microsoft Excel for Microsoft 365 MSO, version 2304) to calculate Overall Dynamic Body Acceleration (ODBA) and Vectorial Dynamic Body Acceleration (VeDBA). The ODBA and VeDBA measures have been used to summarize acceleration previously (Wilson et al. 2006, Shepard et al. 2008, Gleiss et al. 2011, Bidder et al. 2012, DeSantis et al. 2020). Both ODBA and VeDBA were calculated for every recorded frequency (1 Hz), and the average ODBA and VeDBA were calculated for every minute and every 24 hours. When the data loggers were removed from the snakes, most of them had detached from the body wall and were free-floating in the snakes' body cavities. This may have caused fluctuations of baseline VeDBA in this study, however ODBA appeared to be resistant to the free-floating movement and therefore was used to summarize snake movements in this study (Wilson et al. 2019; D. DeSantis, pers. comm.). Boxplots were created in R Studio to compare average 24-hour ODBA on days that snakes experienced harassment and SDT or SDT alone to days that snakes did not experience either treatment. Additionally, boxplots were created to compare average 24-hour ODBA one day after (24-hours) experimentation (i.e., harassment and SDT or SDT alone) to all other days. The data logger for "Steve" was not correctly activated before implantation, so no accelerometry data was obtained for this snake.

For defensive behavior, generalized linear mixed-effects models (Bolker et al. 2009, Found et al. 2018) were constructed using the "glmer" function in the "lme4" package with individual snake ID included as a random effect to account for multiple defensive behavior readings being taken from each individual snake. Fixed effects included treatment, body temperature, days since last treatment, days since experiment began, ambient temperature, substrate temperature, and time of day. Time of day was divided into five categories: 1) 0430-0800 h ("Morning"); 2) after 0800 to 1100 h ("Late Morning"); 3) after 1100 to 1400 h ("Mid-day"); 4) after 1400 to 1700 h ("Afternoon"); 5) after 1700 to 2000 h ("Evening"); following Nowak (2009). Multiple models were created with different fixed effects. Analysis of variance (ANOVA) tests were run to compare the models to each other. Both p-values and Akaike Information Criterion (AIC) are used to assess the "best" model fit for the data, and the use of either test is appropriate (Halsey 2019, Sonderegger 2020). In this study, both AIC and p-values were used to choose the best model fit for the data.

GIS Analyses

All GIS analyses were performed in ArcGIS Pro (Environmental Systems Research Institute, Inc., version 3.0.0) and ArcMap (Environmental Systems Research Institute, Inc., version 10.8.1). Kernel density plots (KDPs) were created using the "Kernel Density" tool in ArcGIS Pro after uploading the coordinated using the "Table to Point" function. The first two weeks of the experiment were excluded from KDP analysis to avoid the dispersion from den sites and post-surgery movements to be included in core use areas. The average distance moved by the snakes per day (44.2 m) was used as the "Search Radius" for each KDP. To calculate the area (in hectares) of core areas for each snake, I used the "Times" tool to multiply each KDP raster by 10,000 and then used the "Int" tool to convert the multiplied raster into integers. I then exported the attribute table for the integer raster into Microsoft Excel. In Excel, I multiplied each cell in the "Value" and "Count" columns and added the above value to each calculation (i.e., Value*Count+ previous (Value*Count)). I used the sum of the "Value*Count" column to calculate proportions (i.e., Proportion=(Count*Value)/Sum). I then found which value was associated with the following proportions: 5%, 25%, 50%, 75%, and 95%. I then divided these values by 10,000 to ensure that the values were the same as the original KDP raster. I then used the “Contour List” tool to use each proportion value to create 5%, 25%, 50%, 75%, and 95% contour lines. I then uploaded the raster with the proportion contour lines to ArcMap and used the “Polylines to Polygons” tool (Jenness Enterprises, Tools for Graphics and Shapes v. 2.1.85) to convert the contour lines to polygons. I used the “Dissolve” tool to dissolve the polygons by contour lines to get the count of each polygon and calculated the hectares of each core area using the “Calculate Geometry” tool in the attribute table of the dissolved raster. The KDPs are presented as “heat maps”, with warmer colors showing locations with a high density of points and cooler colors showing low density of points.

I calculated Minimum Convex Polygons (MCPs) by uploading the coordinates to ArcGIS Pro using the “Table to Point” function. I then used the “Minimum Bounding Geometry” tool to create the MCPs for each snake. I calculated the area (in hectares) of each MCP in the attribute table of the polygon using the “Calculate Geometry” tool.

GIS Data Cleaning

Some data points were removed or altered from the dataset to accurately represent snake movements. Any points that were estimated to be more than 5m away from the actual location of the snake were removed to prevent inaccurate locations. In some cases, the location of the snake was not accessible and UTMs were taken from two locations. In these cases, the UTMs for both locations were averaged to get a general point location for the snake. Post-harassment flight distance calculations for “Jim” on 6/26/22 and 9/2/22 were removed due to the snake’s location being more than 5m from coordinates. Post-flight distance calculations for “Steve” on 4/25/22 and 4/26/22 were removed because the snake was translocated less than 100m, which deviated from protocol. The first two weeks of snake locations were removed from KDP analysis to prevent rapid or migratory movement from den and/or capture sites being included in activity range and 95% core area calculations.

Results

Unfortunately, despite approximately 255 hours of searching for snakes from May 2021 to May 2022, the sample size for this study was very small (n=5, with 3 treatment (harassment and SDT) and 2 "controls" (SDT only)) study subjects. While too small to be able to make any statistically significant comparisons, the patterns from data collected can still be informative to future studies and have important management implications.

Activity Ranges

The mean size of the activity range sizes as estimated by the minimum convex polygon (MCP) for the treatment (harassment and SDT) group was 19.06 hectares (ha) with a standard error of +/- 2.61 ha, and the mean for the control (SDT only) group was 32.60 ha with a standard error of +/- 2.44 ha (Table 3-1). Figures 3-1 through 3-5 show the activity range size for each individual. The mean size of the control group's MCPs were almost double that of the treatment group.

The mean size of the 95% core area kernel density polygons (KDP) for the treatment group was 5.98 ha with a standard error of +/- 0.51 ha, and the mean for the control group was 7.91 ha with a standard error of +/- 1.24 ha (Table 3-2). The mean number of 95% core areas for the treatment group was 10 with a standard error of +/- 2.65, and the mean number of 95% core areas for the control group was 12.5 with a standard error of +/- 2.5. Figures 3-1 through 3-5 show the core areas for each individual. Although no statistical analyses were performed on this data due to a small sample size, the mean size of the control group's 95% core area size and count were larger than that of the treatment group.

Movements

A Shapiro-Wilk test showed that the data for the distance moved per day not including treatment days is not normally distributed (W=0.61249, p=2.2e-16). The data was log-transformed and square root transformed, but the data was still not normal, so non-parametric tests were conducted on this data. The distance moved per day excluding treatment days was not different between the treatment and control group (W=10838, p=0.3815; Table 3-3; Figure 3-6). A Shapiro-Wilk test showed that the data for post-treatment flight distances were normally distributed (W=0.9583, p=0.2983) and a Bartlett's test showed equal variance (Bartlett's K^2=0.8175, p=0.3659). There was no difference in means of post-treatment flight distances between the group that experienced harassment and SDT and group that only experienced SDT (t=-0.2177, p=0.8294; Table 3-4; Figure 3-7), which means that post-treatment flight distances were statistically the same between treatment and control groups.

The average 24-hour ODBA and VeDBA calculations for each snake are presented graphically in Appendix 1 through Appendix 4. Each snake showed variability in their movement patterns, with Ted having the highest mean 24-hour ODBA (Appendix 5). Although no statistical analyses were performed on this data due to small sample size, the mean 24-hour average ODBA did not appear to differ between days that snakes received treatment (i.e., harassment and SDT or SDT alone; x=-0.5320; Appendix 6) and days that snakes did not receive treatment (x=-0.4765; Appendix 6). The mean 24-hour average ODBA also did not appear to differ between the day (24-hours) after experimentation (x=-0.4949; Appendix 7) and all other days (x=-0.4777; Appendix 7).

Body Temperature

The mean body temperature for the group that experienced harassment and SDT was 23.7°C with a standard error of +/- 0.1, and the mean body temperature for the group that only experienced SDT was 25.2°C with a standard error of +/- 0.1 (Table 3-5). Table 3-6 shows the body temperature range for each of the snakes. One snake, Scout, had a noticeably cooler body temperature compared to the other snakes (mean = 22.2°C +/- 0.1; range of 3.6-37.6°C; Table 3-6). A line graph for each treatment group shows the daily mean body temperature for each snake (Figure 3-8), and a scatterplot shows the daily mean body temperature for each snake in each treatment group (Figure 3-9).

Defensive Behavior

There was no significance for any variable included in the defensive behavior models (Table 3-7; Table 3-8), -including treatment (z=1.51, p=0.13), body temperature (z=1.05, p=0.2953), days since experiment began (z=-0.57, p=0.5715), ambient temperature (z=0.02, p=0.981), substrate temperature (z=-0.60, p=0.546), or time of day (Afternoon (Intercept): z=-0.80, p=0.423; Evening: z=0.87, p=0.383; Late morning: z=-1.13, p=0.258; Mid-day: z=-1.23, p=0.218; Morning: z=-0.49, p=0.624). In the model that only included days since last treatment as a variable, there was evidence for a significance (z=1.97, p=0.0489; Table 3-7); there was also significance for this variable in a model including all variables (z=2.43, p=0.0151; Table 3-7). However, in a model that included days since last treatment and treatment group, days since last treatment was not significant (z=1.81, p= 0.0703; Table 3-8; Table 3-8). The small sample size hinders the interpretation of these results. Other than days since last treatment, there was no significance of any variable in a model that included all variables (Treatment: z=1.40, p=0.1631; body temperature: z=0.63, p=0.5320; days since experiment began: z=-0.49, p=0.6275; ambient temperature: z=-0.74, p=0.4594; substrate temperature: z=0.18, p=0.8540; time of day (Evening: z=0.443, p=0.6579; Late morning: z=-1.66, p=0.0974; Mid-day: z=-1.44, p=0.1498; Morning: z=-0.83, p=0.4042); Table 3-8). Time of day did not have any significance in the model that included all variables (X^2=5.60, df=4, p=0.2314; Table 3-8). The variance for all models was 0 or close to 0, signifying that there was no variability in defensive behavior among the five snakes (Table 3-7; Table 3-8; Figure 3-10; Figure 3-11).

Corticosterone Responses

A Shapiro-Wilk test showed that the post-harassment CORT concentrations were normally distributed (W=0.8975, p=0.4188), and a Bartlett's test for homogeneity of variance showed equal variance among groups (Bartlett's K^2=0.8173, p=0.366). Table 3-9 shows that the mean CORT response of the treatment group was 97.15 ng/mL (SE=+/-21.28) and the mean of the control group was 223.47 ng/mL (SE=+/-72.28). The mean CORT response from the control group was more than double that of the treatment group, however, due to the extremely small sample size (n=4), the standard errors were very large. There was no difference between the treatment and control group (t=-1.6766, df=1.172, p=0.3143), which means that the post-harassment CORT responses did not differ between groups. While there is visual evidence that there could be a difference between the groups (Figure 3-12), small sample size precluded statistical analyses.

Biochemical Validations

The results of the accuracy test showed that the observed hormone (CORT) concentration was significantly correlated with the expected hormone (CORT) concentrations (R^2=0.995, slope = 1.235; Figure 2-1; Table 2-2; see Chapter 2). The parallelism test showed that the slopes of the lines for the pooled sample and the CORT standard were not significantly different (i.e., equivalent/parallel) ($F_{1,11}$=0.1348, p=0.7205; Figure 2-2; Table 2-2; see Chapter 2).

Discussion

I compared the spatial ecology, movements, behavior, and physiological responses of *C. atrox* that experienced harassment coupled with short distance translocation (treatment) to the responses of *C. atrox* that only experienced SDT (control). This study was the first, to my knowledge, to investigate the effects of a novel stressor as a management strategy for human-rattlesnake interactions. There was little evidence that spatial ecology, body temperatures, and behaviors of wild *C. atrox* changed as a result of harassment coupled with SDT. Further research and data analyses are needed to determine if harassment coupled with SDT could be used as a management strategy to decrease human-rattlesnake interactions.

It should be noted that the sample size for this study was small (n=5). In some analyses, the sample size was only 4 due to the loss of the study snake “Scout” from trauma to the trachea caused by a bird of prey during early August 2022 (Choi et al. 2022 unpubl.). The accelerometer for the study snake “Steve” was not correctly activated during implantation, therefore no accelerometry data was obtained for this snake. Due to the small sample size, I will be discussing the data patterns and making suggestions based on those patterns. The results from this study should be interpreted with caution, and the impacts of harassment on the spatial ecology, behavior, and physiology in rattlesnakes needs to be further investigated with larger sample sizes.

The home ranges for the control snakes that only experienced SDT were larger than treatment snakes who experienced harassment and SDT. The mean size of home range as estimated by MCPs for the control group were almost double that of the treatment group. Studies have shown that SDT alone increases the size of MCPs in rattlesnakes (Holding et al. 2014a, but see Brown et al. 2009). However, Holding et al. (2014a) did not see a significant increase in

MCPs for *Crotalus oreganus oreganus* that were just captured and handled on a weekly basis; they also determined that multiple instances of translocation did not induce chronic stress in *C. o. oreganus*. The addition of harassment to SDT for the snakes in this study could influence the size of the activity ranges due to increased stress (Heiken et al. 2016). Nowak (2005) found that the average activity range size for male and female *C. atrox* was 12.5 +/- 11.9 Ha at Tuzigoot National Monument. This seems to be consistent with the activity range sizes of the treatment group, but the control group in my study had much larger activity range sizes than what was found in Nowak (2005). The 95% core area KDPs in these studies showed that control snakes had slightly larger KDPs than treatment snakes (~2 ha). The differences in responses between MCP and KDP sizes in this study are likely due to the different techniques used for each home range estimator, with KDPs including areas based on point density versus MCPs drawing boundaries around points (Nilsen et al. 2007). The analysis for KDPs did not include the first two weeks of the study when snakes were dispersing from den sites. Unfortunately, many snake locations had to be removed from analysis for “Jim”, “Gordon”, and “Scout” who were inaccessible due to terrain or property boundaries. These locations are generally included by the MCP boundaries. However, the snakes “Gordon” and “Scout” had many locations in a marsh, which is not represented in their 95% KDPs, and “Jim” had many locations in a fenced off, abandoned water treatment area which is not represented in his 95% KDP. Given this exclusion of multiple snake locations, utility of the KDPs is limited for these snakes. In addition, the sample size does not allow individual variation to be ruled out as a cause for the patterns discussed above.

The distance moved per day excluding treatment days did not differ between the treatment and control groups. This is consistent with the findings of Holding et al. (2014a) that found no differences in distance moved per day in *C. o. oreganus* between control snakes, handled snakes, and translocated snakes. The snakes in this study were located 24-hours post-treatment to evaluate post-treatment flight distances. There was no evidence to suggest that there was a difference in post-treatment flight distances between the treatment and control group. There are studies that have found that rattlesnakes will return to their site of capture after experiencing SDT (Hardy et al. 2001, Brown et al. 2009, Holding et al. 2014a), however there are no studies, to my knowledge, that investigate the flight distance post-translocation or post-harassment. Visually, there was evidence that the average distance moved per day on non-treatment days were lower than the post-treatment flight distances in this study, so it is possible that my results were strongly influenced by sample sizes. The differences between the daily movements and movements in response to treatments will be investigated further in the future to assess possible effects of translocation alone and harassment and translocation on the frequency and distance of movements in this study.

The patterns in the movement data from the accelerometers suggest high variability of movements among each snake with no visible patterns associated with experimentation. The average Vectoral Dynamic Body Acceleration (VeDBA) was graphed in Appendix 1 through Appendix 4, but Overall Dynamic Body Acceleration (ODBA) was chosen for the analyses in this study due to the variability in baseline VeDBA values likely due to the orientation of the data loggers when they dislodged from the body wall and were free-floating in the snakes' body cavities (Wilson et al. 2009; D. DeSantis pers. comm.). There did not appear to be a difference in the average 24-hour ODBA on days that snakes experienced harassment and SDT or SDT alone compared to days that no experimentation occurred. There also did not appear to be a difference in the average 24-hour ODBA on the day (24-hours) after experimentation occurred compared to all other days. The graphs of overall average 24-hour ODBA also show that there did not appear to be a relationship between treatment (either harassment and SDT or SDT alone) on movement patterns in these snakes. High ODBA values have been correlated to high frequencies of movements in studies that have validated movements through field observations (Wilson et al. 2006, Tatler et al. 2018). However, ODBA is only used as a proxy for movement frequency and some behaviors and movements are incorrectly represented by high ODBA values (Tatler et al. 2018). Species- and study site-specific behavior models derived from field observation validations are required to further classify the movements in this study (Tatler et al. 2018, DeSantis et al. 2020), and will be investigated for my study subjects in the future. With study-specific behavior models, we could investigate the effects that the harassment method has on the accelerometry data, the impact of harassment coupled with SDT on the behaviors exhibited in these snakes (i.e., more or less time spent foraging), and the number of intentional movements per day (i.e., flight behaviors), for snakes that experienced harassment and SDT compared to snakes that only experienced SDT. More studies on the effects of harassment on movement patterns are needed to fully understand the implications of these patterns in *C. atrox*.

Body temperatures did not differ greatly among individuals or between the treatment and control groups. The treatment group had slightly lower average daily body temperatures, perhaps influenced by “Scout”, who had the lowest daily average body temperature. The low body temperatures in this snake could be influenced by his use of a marsh early in the season when ambient and water temperatures were cooler. The treatment snake “Gordon” had the highest average daily body temperature. We wanted to investigate body temperature as a proxy for habitat use (i.e., it is possible that harassed and translocated snakes could be underground more often and therefore colder; Lelièvre et al. 2011); however, treatment and control groups showed similar body temperatures and therefore similar time spent above and below ground. Holding et al. (2014b) only detected an effect of translocation on body temperature the first week of translocation in *C. o. oreganus* and saw no impacts of the treatment for the remaining 5 weeks of the experiment. Heiken (2013) and Roe et al. (2010) also found that body temperature was not affected by translocation, however these studies were investigating the effects of LDT (long distance translocation). The results of this study appear to be consistent with the effects of translocation on body temperature in other studies. Both average daily body temperatures and body temperature ranges for each snake were presented in this study, however further analyses on the optimal body temperature for each snake (reviewed in Angilletta et al. 2002) will be conducted to better understand the effects of harassment on body temperatures in this study.

Defensive behavior did not differ between treatment and control groups in this study. Pitvipers have not exhibited changes in defensive behavior in response to post-harassment stress (Herr et al. 2017) or elevated CORT levels (Claunch et al. 2017). Extra experimental effects also did not influence defensive behavior in this study including body temperature, ambient temperature, substrate temperature, time of day, or days since experiment began. Body temperatures have been shown to increase likelihood of strikes (Whiteford et al. 2020); however, that study purposefully elicited a defensive response in multiple *Crotalus* species in a lab setting, whereas my study focused on unprovoked initial defensive behavior in a field setting. Kissner et al. (1997) did not find a correlation between body temperature and rattling in *C. viridis viridis*, but studies in other species found that warmer snakes were more likely to rattle when approached (Prior and Weatherhead 1994, Rowe and Owings 1996). Ambient and substrate temperature likely showed no effect since body temperature is commonly correlated with ambient temperature (Mushinsky et al. 1980). Time of day and ambient temperature are also related, which showed no effect on defensive behavior. There was no difference in defensive behavior in treatment or control groups as the experiment progressed over time (i.e., days since experiment began). The snakes did not become more or less defensive when they experienced more treatments, which is consistent with the findings of Herr et al. (2017) that stress does not increase the likelihood of defensive behavior. The variable of "days since last treatment" showed possible significance in the defensive behavior model when treatment group was excluded, possibly due to a lack of variability in the defensive behavior responses and a small sample size. However, in a model that also included treatment group as a variable, "days since last treatment" lost its significance. It is possible that the number of days since the snake experienced treatment may have had an effect on defensive behavior, but this variable will need to be investigated with a larger sample size. It is possible that there were no patterns found in defensive behavior in this study because snakes did not show initial defensive behaviors most of the time. This is not unexpected, since the primary predator avoidance behavior in rattlesnakes is crypsis (Nowak and Greene 2016). There was also no variability among snakes in the defensive behavior model. Viperid snakes show individual personalities in their defensive behavior (Gibert et al. 2022), and the lack of variation among the snakes in the current defensive behavior model is likely due to the small sample size and lack of initial defensive behavior in all snakes.

There was no statistical difference between plasma CORT concentrations post-harassment between treatment and control snakes at the end of the study. However, visual representation of the data shows that treatment group snakes had a higher response to harassment, with the mean CORT concentration being more than double that of the control group. The purpose of taking post-harassment CORT from both groups was to investigate if the treatment group snakes became habituated to the harassment method (i.e., less severe CORT response). Compared to the treatment group, it appears that the control group did not have as severe a CORT reaction to the harassment method. Based on the differences between these two groups, it does not appear that the treatment group became habituated to the harassment method. The sample size for this analysis was only 4 snakes, so further analysis on the difference between treatment and control group post-harassment CORT levels is needed. Another variable that should be considered in this context is baseline CORT levels over time. We were not able to obtain baseline CORT concentrations before the study began due to our surveying strategies. Like Holding et al. (2014a) and Heiken et al. (2016), baseline CORT concentrations should be taken before experimentation begins to compare them to CORT concentrations when the experiment ends. Increased levels of baseline plasma CORT concentrations can be an indicator of chronic stress (Moore et al. 1991, Jones and Bell 2004, Sykes and Klukowski 2009). The goal of this study was to find a human-rattlesnake management strategy that does not cause chronic stress in rattlesnakes. It is important to investigate the effects of baseline CORT concentrations from this harassment method before it is used in a management setting.

In this study, there was little evidence that behaviors, spatial ecology, or physiology were affected by harassment coupled with SDT. Additional research and data analyses are needed to further understand if this method could lead to human avoidance and learning in rattlesnakes. Although the sample size in this study was small, the patterns in the data could be informative to future studies investigating the effectiveness of harassment as a management strategy. We know that the harassment strategy successfully raises plasma CORT in *C. atrox* (see Chapter 2). Studies have shown that reptiles are able to change their behaviors to avoid novel stressors (Thaker et al. 2009, 2010; Trompeter and Langkilde 2011; Lauger 2019) and pitvipers have the ability to learn (Krochmal et al. 2018). Using multiple instances of a repeated stressor has also been effective in changing behaviors in other animals (Rauer et al. 2003, Kloppers et al. 2005, Mazur 2010, Found et al. 2018, Found and St. Claire 2018). With further research, the results from this study could be used to inform future studies to deter rattlesnakes in high-traffic public use areas (e.g., around dwellings, along trails) within areas such as national parks and monuments where other management strategies such as habitat modification are unavailable, as well as in other public areas by associating humans and areas used by humans with a negative stimulus. Once the impacts on snake health, body condition, and stress are investigated more thoroughly, the method in this study could potentially be used by snake removal companies to deter repeated instances of snakes at private residences. Professionals and volunteers could be safely trained (e.g., Nowak and Green 2016) to properly inflict the harassment method followed by SDT on snakes that are present in areas where possible human-rattlesnake interactions are high. Training could be coupled with educational programs informing the public about rattlesnake behavior and how to best reduce the chances of venomous snakebite (e.g., Nowak and Green 2016). Managing human-rattlesnake interactions is crucial to environmental and public health.

LITERATURE CITED

Amarello, M., E.M. Nowak, E.N. Taylor, G.W. Schuett, R.A. Repp, P.C. Rosen, and D.L. Hardy, Sr. 2010. Potential environmental influences on variation in body size and sexual dimorphism among Arizona populations of the western diamond-backed rattlesnake (*Crotalus atrox*). Journal of Arid Environments 74: 1443-1449.

Angilletta, M.J., P.H. Niewiarowski, and C.A. Navas. 2002. The evolution of thermal physiology in ectotherms. Journal of Thermal Biology 27: 249-268.

Bailey, F.C., V.A. Cobb, T.R. Rainwater, T. Worrall, and M. Kluwowski. 2009. Adrenocortical effects of human encounters of free-ranging Cottonmouths (*Agkistrodon piscivorus*). Journal of Herpetology 43: 260-266.

Bidder, O.R., L.A. Qasem, and R.P. Wilson. 2012. On higher ground: how well can dynamic body acceleration determine speed in variable terrain? PLoS ONE 10: e0139351.

Bolker, B.M., M.E. Brooks, C.J. Clark, S.W. Geange, J.R. Poulsen, M.H.H. Stevens, and J.S.S. White. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends in Ecology and Evolution 24(3): 127-135.

Brown, J., C. Bishop, and R. Brooks. 2009. Effectiveness of short-distance translocation and its effects on western rattlesnakes. Journal of Wildlife Management 73: 419-425.

Brown, T.K., J.M. Lemm, J. Montagne, J.A. Tracey, and A.C. Alberts. 2011. Spatial ecology, habitat use, and survivorship of resident and translocated Red Diamond Rattlesnakes (*Crotalus ruber*). *In* W. K. Hayes, K. R. Beaman, M.D. Cardwell, and S. P. Bush (eds.), The Biology of the Rattlesnakes, pp. 377-394. Loma Linda University Press, Loma Linda, CA.

Burghardt, G.M., H.C. Wilcoxon, and J.A. Czaplicki. 1973. Conditioning in garter snakes: aversion to palatable prey induced by delayed illness. Animal Learning and Behavior 1: 317-320.

Campbell, J.A., and W.W. Lamar. 2004. The venomous reptiles of the Western Hemisphere, Volume 2. Cornell University Press (Comstock Publishing Associates), Ithaca, New York, USA.

Capehart, G.D., C. Escallon, B.J. Vernasco, I.T. Moore, and E.N. Taylor. 2016. No drought about it: effects of supplemental hydration on the ecology, behavior, and physiology of free-ranging rattlesnakes. Journal of Arid Environments 134: 79-86.

Choi, E., M.K. Keel, K.E. O'Connell, and M. Schlesinger. 2022. Pathology 22N1675 Gross Report. Unpublished Necropsy Report, University of California, Davis, California.

Claunch, N.M., J.A. Frazier, C. Escallon, B.J. Vernasco, I.T. Moore, and E.N. Taylor. 2017. Physiological and behavioral effects of exogenous corticosterone in a free-ranging ectotherm. General and Comparative Endocrinology 248: 87-96.

Cocks, K, E.M. Nowak, and C. Calvo. 2019. Does anthropogenic disturbance influence defensive behavior of *Crotalus atrox* in national monuments. Biology of Pitvipers Symposium 3. Rodeo, NM.

Corneille, M.G., S. Larson, R.M. Stewart, D. Dent, J.G. Myers, P.P. Lopez, M.J. McFarland, and S.M. Cohn. 2006. A large single-center experience with treatment patients with crotalid envenomations: outcomes with and evolution of antivenin therapy. American Journal of Surgery 192: 848-852.

Dart, R. C., J.T. McNally, D. W. Spaite, and R. Gustafson. 1992. The sequelae of pitviper poisoning in the United States. Pp. 395-404 *In* J.A. Campbell and E.D. Brodie, Jr. (Eds.), Biology of the Pitvipers, Selva, Tyler, Texas.

DeSantis, D.L., V. Mata-Silva, J.D. Johnson, and A.E. Wagler. 2020. Integrative framework for long-term activity monitoring of small and secretive animals: Validation with a cryptic pitviper. Frontiers in Ecology and Evolution 8: 169.

Engel, T.R. 1997. Seed dispersal and plant regeneration by snakes? Ecotropica 3: 33-41.

Found, R., and C.C. St. Claire. 2018. Personality influences wildlife responses to aversive conditioning. The Journal of Wildlife Management 82: 747-755.

Found, R., E.L. Kloppers, T.E. Hurd, and C.C. St. Claire. 2018. Intermediate frequency of aversive conditioning best restores wariness in habituated elk (*Cercus canadensis*). PLoS One 13: e0199216. https://doi.org/10.1371/journal.pone.0199216.

French, S.S., H.B. Fokidis, and M.C. Moore. 2008. Variation in stress and innate immunity in the Tree Lizard (*Urosaurus ornatus*) across an urban-rural gradient. Journal of Comparative Physiology 178: 997-1005.

Gibert, R.G., D.W. Maag, L.N. Sanders, and R.W. Clark. 2022. Investigating personality in vipers: individual rattlesnakes exhibit consistent behavioral responses in defensive and exploratory contexts. Behavioral Ecology and Sociobiology 76: 132.

Gleiss, A.C., R.P. Wilson, and E.L. Shepard. 2011. Making overall dynamic body acceleration work: on the theory of acceleration as a proxy for energy expenditure. Methods in Ecology and Evolution 2: 23-33.

Greene, H.W. 1997. Snakes. The Evolution of Mystery in Nature. University of California Press, Berkley, California.

Greene, H.W. 2013. Tracks and Shadows. Field Biology as Art. University of California Press, Berkeley, California.

Greene, H.W. and J.A. Campbell. 1992. The future of pitvipers. Pp. 421-427 *In* J.A. Campbell and ED. Brodie (Eds.), Biology of the Pitvipers. Selva Press, Tyler, Texas.

Halsey, L.G. 2019. The reign of the p-value is over: what alternative analyses could we employ to fill the power vacuum? Biology Letters 15: 20190174.

Hardy, D.L. and H.W. Greene. 1999a. Borderland black-tails: radiotelemetry, natural history, and living with venomous snakes. Pp. 117-121 *In* G.J. Gottfried, L.G. Eskew, C.G. Curtin, and C.B. Edminister (Eds.), Toward Integrated Research, Land Management, and Ecosystem Protection in the Malpais Borderlands: Conference Summary #RMRS-P-10, USDA Forest Service, Rocky Mountain Research Station, Fort Collins, Colorado.

Hardy, D.L. and H.W. Greene. 1999b. Surgery on rattlesnakes in the field for implantation of transmitters. Sonoran Herpetology 12: 26-28.

Hardy, D.L., Sr., H.W. Greene, B. Tomberlin, and M. Webster. 2001. Relocation of nuisance rattlesnakes: problems using short-distance translocation in a small rural community. Pp. 44-45 *In* W.L. Halvorson and B.S. Gebow (Eds.), Creative Cooperation in Resource Management: Third Conference on Research and Resource Management in the Southwest Desert, Extended Abstracts. USGS Sonoran Desert Field Station, University of Arizona, Tucson, Arizona.

Hawley, J.E., T.M. Gehring, R.N. Schultz, S.T. Rossler, and A.P. Wydeven. 2009. Assessment of shock collars as nonlethal management for wolves in Wisconsin. Journal of Wildlife Management 73: 518-525.

Heiken, K.H. 2013. The behavioral and physiological effects of long-distance translocation on Western Rattlesnakes (*Crotalus oreganus*). Unpublished Master’s Thesis, California Polytechnic State University, San Luis Obispo, California, USA.

Heiken, K.H., G.A. Brusch, S. Gartland, C. Escallon, I.T. Moore, and E.N. Taylor. 2016. Effects of long distance translocation on corticosterone and testosterone levels in male rattlesnakes. 2016. General and Comparative Endocrinology 237: 27-33.

Herr, M.W., S.P. Graham, and T. Langkilde. 2017. Stressed snakes strike first: Hormone levels and defensive behavior in free ranging cottonmouths (*Agkistrodon piscivorus*). General and Comparative Endocrinology 243: 89-95.

Holding, M.L, D.A.S. Owen, and E.N. Taylor. 2014b. Evaluating the thermal effects of translocation in a large-bodied pitviper. JEZ-A Ecological and Integrative Physiology 321(8): 442-449.

Holding, M.L., J.A. Frazier, S.W. Dorr, S.N. Henningsen, I.T. Moore, and E.N. Taylor. 2014a. Physiological and behavioral effects of repeated handling and short-distance translocation on free-ranging Northern Pacific Rattlesnakes (*Crotalus oreganus oreganus*). Journal of Herpetology 48: 233-239.

Hollander, M., D.A. Wolfe, and E. Chicken. 2013. Nonparametric statistical methods. John Wiley & Sons, Hoboken, New Jersey.

Jones, S.M, and K. Bell. 2004. Plasma corticosterone concentrations in males of the skink *Egernia whitii* during acute and chronic confinement and over a diel period. Comparative Biochemistry and Physiology 137: 105-113.

Kissner, K.J., M.R. Forbes, and D.M. Secoy. 1997. Rattling behavior of Prairie Rattlesnake (*Crotalus viridis viridis*, Viperidae) in relation to sex, reproductive status, body size, and body temperature. Ethology 103: 1042-1050.

Klauber, L.M. 1972. Rattlesnakes: Their Habits, Life Histories, and Influence on Mankind, 2 Vols., 2nd ed. University of California Press, Berkeley and Los Angeles.

Kloppers, E.L., C. C. St. Clair, and T.E. Hurd. 2005. Predator-Resembling Aversive Conditioning for Managing Habituated Wildlife. Ecology and Society 10(1): 31.

Krochmal, A.R., A.J. Place, T.J. LaDuc, and T.C. Roth II. 2018. Phylogenetic patterns in learning and decision making in pit vipers (Viperidae: Crotalinae). Animal Behaviour 145: 117-123.

Langkilde, T. and R. Shine. 2006. How much stress do researchers inflict on their study animals? A case study using a scincid lizard, *Eulamprus heatwolei*. Journal of Experimental Biology 209: 1035-1043.

Lauger, K.K. 2019. Stress Physiology, Behavior, and Growth of Federally Threatened Narrow-headed Gartersnakes in Captivity. Unpublished Master's Thesis, Northern Arizona University, Flagstaff, Arizona, USA.

Lelièvre, H., G. Blouin-Demers, D. Pinaud, H. Lisse, X. Bonnet, and O. Lourdais. 2011. Contrasted thermal preferences translate into divergences in habitat use and realized performance in two sympatric snakes. Journal of Zoology 284(4): 265-275.

Mazur, R.L. 2010. Does aversive conditioning reduce human-black bear conflict? Journal of Wildlife Management 74: 48-54.

McDiamrmid, R.W., M.S. Foster, C. Guyer, J.W. Gibbons, and N. Chernoff. 2012. Reptile Biodiversity: Standard Methods for Inventory and Monitoring. University of California Press, Berkley and Los Angeles, California.

Minckley, W.L. and D.E. Brown. 1994. Tropical-subtropical wetlands. Pp. 268-287 *In* D.E. Brown (Ed.), Biotic Communities of the Southwestern United States and Mexico. University of Utah Press, Salt Lake City.

Moeller, K.T., G. Demare, S. Davies, and D.F. DeNardo. 2017. Dehydration enhances multiple physiological defense mechanisms in a desert lizard, *Heloderma suspectum*. Journal of Experimental Biology 220: 2166-2174.

Moore, M.C., C.W. Thompson, and C.A. Marler. 1991. Reciprocal changes in corticosterone and testosterone levels following acute and chronic handling stress in the Tree Lizard, *Urosaurus ornatus*. General and Comparative Endocrinology 81: 217-226.

Moynihan, J.A. 2003. Mechanisms of stress-induced modulation of immunity. Brain, Behavior, and Immunity 17: S11-S16.

Mushinsky, H.R., J.J. Hebrard, and M.G. Walley. 1980. The role of temperature on the behavioral and ecological associations of sympatric water snakes. Copeia 1980(4): 744-754.

National Park Service. 2022. Annual visitation by park. https://irma.nps.gov/Stats/SSRSReports/Park%20Specific%20Reports/Annual%20Park%20Recreation%20Visitation%20(1904%20-%20Last%20Calendar%20Year)?Park=TUZI; Accessed 4, January 2023

Nilsen, E.B., S. Pedersen, and J.D. Linnell. 2007. Can minimum convex polygon home ranges be used to draw biologically meaningful conclusions? Ecological Research 23: 635-639.

Nowak, E.M. 1998. Effects and effectiveness of rattlesnake relocation at Montezuma Castle National Monument, Arizona. Unpublished Master's Thesis, Northern Arizona University, Flagstaff.

Nowak, E.M. 2005. Movement patterns and life history of wester diamond-backed rattlesnakes (*Crotalus atrox*) at Tuzigoot National Monument, Arizona. Pp. 235-274 *In* C. van Riper III and D. Mattson, (Eds.), The Colorado Plateau II: Biophysical, socioeconomic, and cultural research. University of Arizona Press, Tucson, Arizona.

Nowak, E.M. 2009. Ecology and management of venomous reptilian predators. Unpublished PhD Dissertation, Northern Arizona University, Flagstaff, Arizona, USA.

Nowak, E.M. and C. van Riper III. 1999. Effects and effectiveness of rattlesnake relocation at Montezuma Castle National Monument. U.S. Geological Survey/FRESC Report Series USGS/FRESC/COPL/1999/17.

Nowak, E.M. and H.W. Greene. 2016. Rattlesnake Conservation in the 21st Century. Pp 413-458 *In* G.W. Schuett, M.J. Feldner, C.F. Smith, and R.S. Reiserer (Eds.), Rattlesnakes of Arizona, Volume 2: Conservation, Behavior, Venom, and Evolution. ECO Herpetological Publishing, Rodeo, New Mexico.

Nowak, E.M., T. Hare, and J. McNally. 2002. Management of "nuisance" vipers: effects of translocation on western-diamond-backed rattlesnakes (*Crotalus atrox*). Biology of the Vipers 34: 533-560.

Nowak, E.M., T.C. Theimer, and G.W. Schuett. 2008. Functional and numerical responses of predators: Where do vipers fit in the traditional paradigms? Biological Review 83: 601-620.

Paradis, S., and M. Cabanac. 2004. Flavor aversion learning induced by lithium chloride in reptiles but not in amphibians. Behavioral Processes 67: 11-18.

Peterson, C.E., and J.B. Sealy. 2021. Threats and Conservation Status. Pp. 81-98 *In* Breisch, A.R., W.H. Martin, J.B. Sealy, C.E. Peterson, and E. Possardt (Eds). The Timber Rattlesnake: Life History, Distribution, Status, and Conservation Action Plan. Partners in

Amphibian and Reptile Conservation Technical Publication CAP-1. Published by the Amphibian and Reptile Conservancy, Nashville, Tennessee.

Prior, K.A., and P.J. Weatherhead. 1994. Response of free-ranging Eastern Massasauga rattlesnakes to human disturbance. Journal of Herpetology 28: 255-257.

Rauer, G., P. Kaczensky, and F. Knauer. 2003. Experiences with aversive conditioning of habituated brown bears in Austria and other European countries. Ursus 14: 215-224.

Reinert, H.K. 1992. Radiotelemetric field studies of pitvipers: data acquisition and analysis. Pp. 185-198 *In* J.A. Campbell and E.D. Brodie, Jr., (Eds.), Biology of the Pitvipers. Selva, Tyler, Texas.

Reinert, H.K., and R.R. Rupert. 1999. Impacts of translocation on behavior and survival of timber rattlesnakes, *Crotalus horridus*. Journal of Herpetology 33: 45-61.

Reiserer, R.S., G.W. Schuett, and H.W. Greene. 2018. Seed ingestion and germination in rattlesnakes: overlooked agents of rescue and secondary dispersal. Proceedings of the Royal Society B: Biological Sciences 285: 20172755.

Roe, J.H., M.R. Frank, S.E. Gibson, O. Attum, and B.A. Kingsbury. 2010. No place like home: an experimental comparison of reintroduction strategies using snakes. Journal of Applied Ecology 47(6): 1253-1261.

Romero, L.M, and J.M. Reed. 2005. Collecting baseline corticosterone samples in the field: is under 3 min good enough? Comparative Biochemistry and Physiology, Part A 140: 73-39.

Rowe, M.P., and D.H. Owings. 1996. Probing, assessment and management during interactions between ground squirrels (Rodentia: Sciuridae) and rattlesnakes (Squamata: Viperidae). 2: Cues afforded by rattlesnake rattling. Ethology 102: 856-874.

Sapolsky, R.M., L.M. Romero, and A.U. Munck. 2000. How do glucocoirticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocrine Reviews 21: 55-89.

Shepard, E.L, R.P. Wilson, F. Quintana, A. Gómez Laich, N. Liebsch, D.A. Albareda, L.G. Halsey, A. Gleiss, D.T. Morgan, A.E. Myers, C. Newman, and D.W. Macdonald. 2008. Identification of animal movement patterns using tri-axial accelerometry. Endangered Species Research 10: 47-60.

Sonderegger, D.L. 2020. Statistical Methods II. Bookdown.org. https://bookdown.org/dereksonderegger/571/.

Sonderegger, D.L., and R. Buscaglia. 2020. Introduction to Statistical Methodology, Second Edition. Bookdown.org. https://bookdown.org/dereksonderegger/570/.

Sullivan, B.K., D.J. Leavitt, and K.O.Sullivan. 2017. Snake communities on the urban fringe in the Sonoran Desert: influences on species richness and abundance. Urban Ecosystems 20: 199-206.

Sullivan, B.K., E.M. Nowak, and M.A. Kwiatkowski. 2014. Mitigation translocation of nuisance herpetofauna: can it be improved? Conservation Biology 29: 12-18.

Sun, L., R. Shine, Z. Debi, T. Zhengren. 2001. Biotic and abiotic influences on activity patterns of insular pit-vipers (*Gloydius shedaoensis*, Viperidae) from north-eastern China. Biological Conservation 97: 387-398.

Sykes, K.L., and M. Klukowski. 2009. Effects of acute temperature change, confinement, and housing on plasma corticosterone in water snakes, *Nerodia sipedon* (Colubridae: Natricinae). Journal of Experimental Zoology 311: 172-181.

Tatler, J., P. Cassey, and T.A.A. Prowse. 2018. High accuracy at low frequency: detailed behavioral classification from accelerometer data. Journal of Experimental Biology 221: jeb184085.

Taylor, E.N. and W. Booth. 2016. Rattlesnakes as Models for Reproductive Studies of Vertebrates. Pp 121-157 *In* G.W. Schuett, M.J. Feldner, C.F. Smith, and R.S. Reiserer (Eds.), Rattlesnakes of Arizona, Volume 2: Conservation, Behavior, Venom, and Evolution. ECO Herpetological Publishing, Rodeo, New Mexico.

Taylor, E.N., D.F. DeNardo, and M.A. Malaway. 2004. A comparison between point- and semi-continuous sampling for assessing body temperature in a free-ranging ectotherm. Journal of Thermal Biology 29: 91-96.

Thaker, M., A.T. Vanak, S.L. Lima, and D.K. Hews. 2010. Stress and aversive learning in a wild vertebrate: the role of corticosterone in mediating escape from a novel stressor. The American Naturalist 175: 50-60.

Thaker, M., S. Lima, and D.K. Hews. 2009. Acute corticosterone elevation enhances antipredator behaviors in male tree lizard morphs. Hormones and Behavior 56: 51-57.

Tokish, J.T., J. Benjamin, and F. Walter. 2001. Crotalid envenomation: the southern Arizona experience. Journal of Orthopaedic Trauma 15: 5-9.

Trompeter, W.P., and T. Langkilde. 2011. Invader danger: lizards faced with novel predators exhibit an altered behavioral response to stress. Hormones and Behavior 60: 152-158

Turner, R.M. and D.E. Brown. 1994. Tropical-subtropical desertland. Pp. 180-221 *In* D.E. Brown (Ed.), Biotic Communities of the Southwestern United States and Mexico. University of Utah Press, Salt Lake City.

Ward-Fear, G., J. Thomas, J.K. Webb, D.J. Pearson, and R. Shine. 2017. Eliciting conditioned taste aversion in lizards: live toxic prey are more effective than scent and taste cues alone. Integrative Zoology 12: 112-120.

Whiteford, M.D., G.A. Freymiller, T.E. Higham, and R.W. Clark. 2020. The effects of temperature on the defensive strikes of rattlesnakes. Journal of Experimental Biology 223(14): jeb223859.

Wilson, R.P., C.R. White, F. Quintana, L.G. Halsey, N. Liebsch, G.R. Martin, and P.J. Butler. 2006. Moving towards acceleration for estimates of activity-specific metabolic rate in free-living animals: the case of the cormorant. Journal of Animal Ecology 75: 1081-1090.

Wilson, R.P., L. Börger, M.D. Holton, D.M. Scantlebury, A. Gómez-Laich, F. Quintana, F. Rosell, P.M. Graf, H. Williams, R. Gunner, L. Hopkins, N. Marks, N.R. Geraldi, C.M. Duarte, R. Scott, M.S. Strano, H. Robotka, C. Eizaguirre, A. Fahlman, and E.L.C. Shepard. 2019. Estimates for energy expenditure in free-living animals using acceleration proxies: A reappraisal. Journal of Animal Ecology 89: 161-172.

TABLE CAPTIONS

TABLE 3-1. Home range sizes as estimated by the minimum convex polygon (MCP) method for five adult male Western Diamond-backed Rattlesnakes (*Crotalus atrox*) telemetered at Tuzigoot National Monument, Yavapai County, Arizona from April 2022 to September 2022. The snakes were opportunistically treated no more than once a week with either harassment and short distance translocation ("treatment") or short distance translocation without harassment ("control"). Table includes snake name, treatment group, mean and standard error for each treatment group, and total size of each snake's activity range in hectares.

TABLE 3-2. Kernel density plot (KDP) 95% core area sizes for five adult male Western Diamond-backed Rattlesnakes (*Crotalus atrox*) telemetered at Tuzigoot National Monument, Yavapai County, Arizona from April 2022 to September 2022. The snakes were opportunistically treated no more than once a week with either harassment and short distance translocation ("treatment") or short distance translocation without harassment ("control"). Table includes snake name, treatment group, number and total size of 95% core areas for each snake, mean and standard error for the number ("count") of 95% core areas for each treatment group, and the mean and standard error of 95% core areas for each treatment group.

TABLE 3-3. Mean and standard error of the average distance moved per day not including treatment days (in meters) for each individual snake and each treatment group for five adult male Western Diamond-backed Rattlesnakes (*Crotalus atrox*) telemetered at Tuzigoot National Monument, Yavapai County, Arizona from April 2022 to September 2022. The snakes were opportunistically treated no more than once a week with either harassment and short distance translocation ("treatment") or short distance translocation without harassment ("control").

TABLE 3-4. Distance moved twenty-four hours after treatment by five adult male Western Diamond-backed Rattlesnakes (*Crotalus atrox*) telemetered at Tuzigoot National Monument, Yavapai County, Arizona from April 2022-September 2022. The snakes were opportunistically treated no more than once a week with either harassment and short distance translocation ("treatment") or short distance translocation without harassment ("control"). Post-treatment flight distances did not include the distance the snakes were translocated. The table includes snake name, treatment group, date of treatment, date twenty-four hours post-harassment, snake movement ("Y" = snake moved within the 24-hours post-treatment, "N" = snake did not move within the 24-hours post-treatment), and the distance moved in meters.

TABLE 3-5. Body temperature data for five adult male Western Diamond-backed Rattlesnakes (*Crotalus atrox*) telemetered at Tuzigoot National Monument, Yavapai County, Arizona from April 2022-September 2022. The snakes were opportunistically treated no more than once a week with either harassment and short distance translocation ("treatment") or short distance translocation without harassment ("control"). The table includes snake name, the mean daily body temperature for each snake in degrees Celsius with standard error, treatment group (treatment or control), and the mean daily body temperature in degrees Celsius with standard error for each treatment group.

TABLE 3-6. Body temperature ranges for five adult male Western Diamond-backed Rattlesnakes (*Crotalus atrox*) telemetered at Tuzigoot National Monument, Yavapai County, Arizona from April 2022 to September 2022. The snakes were opportunistically treated no more than once a week with either harassment and short distance translocation (“treatment”) or short distance translocation without harassment (“control”). The table includes snake name, the treatment group for each snake (treatment or control), and the body temperature range in degrees Celsius for each snake.

TABLE 3-7. Logistic regression models examining defensive behavior of five telemetered Western Diamond-backed Rattlesnakes (*Crotalus atrox*) from Tuzigoot National Monument, Yavapai County, Arizona from April 2022 to September 2022 as it relates to treatment, body temperatures, days since last treatment, days since experiment began, ambient temperature, substrate temperature, and time of day. Snake ID was included as a random effect to account for the fact that multiple samples were taken from the same snake. The snakes were opportunistically treated no more than once a week with either harassment and short distance translocation (“treatment”) or short distance translocation without harassment (“control”). Model number and model variables, Akaike Information Criteria (AIC), the variance of the random effects, and the resulting p-values for each test were given.

TABLE 3-8. Logistic regression models examining defensive behavior of five telemetered Western Diamond-backed Rattlesnakes (*Crotalus atrox*) from Tuzigoot National Monument, Yavapai County, Arizona from April 2022 to September 2022 as it relates to treatment, body temperatures, days since last treatment, days since experiment began, ambient temperature, substrate temperature, and time of day. Snake ID was included as a random effect to account for the fact that multiple samples were taken from the same snake. The snakes were opportunistically treated no more than once a week with either harassment and short distance translocation (“treatment”) or short distance translocation without harassment (“control”). Model number and model variables, Akaike Information Criteria (AIC), the variance of the random effects, the resulting p-values for each test, models included in Analysis of Variance (ANOVA), and the p-value for the ANOVA were given.

TABLE 3-9. Plasma corticosterone (CORT) levels sampled from four adult male Western Diamond-backed Rattlesnakes (*Crotalus atrox*) telemetered at Tuzigoot National Monument in Yavapai County, Arizona during September 2022 following harassment. During telemetry between April and September 2022, the snakes were opportunistically treated no more than once a week with either harassment and short distance translocation (“treatment”) or short distance translocation without harassment (“control”). The snake’s name, treatment group, plasma corticosterone (CORT) concentration (in ng/mL), mean CORT concentration (in ng/mL) for each treatment group, and standard errors are given.

FIGURE LEGENDS

FIGURE 3-1. Home range and core areas as estimated by A) activity range as estimated by the minimum convex polygon (MCP) and B) kernel density plot (KDP) created in ArcGIS Pro for the adult male Western Diamond-backed Rattlesnake (*Crotalus atrox*) “Gordon” at Tuzigoot National Monument in Yavapai County, Arizona who was telemetered from April 2022 to September 2022. The KDPs are represented as heat maps with warmer colors indicating a higher density of locations and cooler colors indicating a lower density of locations. “Gordon” was opportunistically treated no more than once a week with harassment and short distance translocation (“treatment”).

FIGURE 3-2. Home range and core areas as estimated by A) activity range as estimated by the minimum convex polygon (MCP) and B) kernel density plot (KDP) created in ArcGIS Pro for the adult male Western Diamond-backed Rattlesnake (*Crotalus atrox*) “Scout” at Tuzigoot National Monument in Yavapai County, Arizona who was telemetered from April 2022 to September 2022. The KDPs are represented as heat maps with warmer colors indicating a higher density of locations and cooler colors indicating a lower density of locations. “Scout” was opportunistically treated no more than once a week with harassment and short distance translocation (“treatment”).

FIGURE 3-3. Home range and core areas as estimated by A) activity range as estimated by the minimum convex polygon (MCP) and B) kernel density plot (KDP) created in ArcGIS Pro for the adult male Western Diamond-backed Rattlesnake (*Crotalus atrox*) “Ted” at Tuzigoot National Monument in Yavapai County, Arizona who was telemetered from April 2022 to September 2022. The KDPs are represented as heat maps with warmer colors indicating a higher density of locations and cooler colors indicating a lower density of locations. “Ted” was opportunistically treated no more than once a week with harassment and short distance translocation (“treatment”).

FIGURE 3-4. Home range and core areas as estimated by A) activity range as estimated by the minimum convex polygon (MCP) and B) kernel density plot (KDP) created in ArcGIS Pro for the adult male Western Diamond-backed Rattlesnake (*Crotalus atrox*) “Jim” at Tuzigoot National Monument in Yavapai County, Arizona who was telemetered from April 2022 to September 2022. The KDPs are represented as heat maps with warmer colors indicating a higher density of locations and cooler colors indicating a lower density of locations. “Jim” was opportunistically treated no more than once a week with short distance translocation without harassment (“control”).

FIGURE 3-5. Home range and core areas as estimated by A) activity range as estimated by the minimum convex polygon (MCP) and B) kernel density plot (KDP) created in ArcGIS Pro for the adult male Western Diamond-backed Rattlesnake (*Crotalus atrox*) “Steve” at Tuzigoot National Monument in Yavapai County, Arizona who was telemetered from April 2022 to September 2022. The KDPs are represented as heat maps with warmer colors indicating a higher density of locations and cooler colors indicating a lower density of locations. “Steve” was opportunistically treated no more than once a week with short distance translocation without harassment (“control”).

FIGURE 3-6. Distance moved per day excluding treatment days by treatment group for five adult male telemetered Western Diamond-backed Rattlesnakes (*Crotalus atrox*) at Tuzigoot National Monument in Yavapai County, Arizona from April 2022 to September 2022. The snakes were opportunistically treated no more than once a week with either harassment and short distance translocation (“treatment”) or short distance translocation without harassment (“control”). The bars are color coded by treatment groups: blue for treatment and orange for control. The graphs include standard error bars.

FIGURE 3-7. Twenty-four-hour post-treatment flight distance by treatment group for five adult male telemetered Western Diamond-backed Rattlesnakes (*Crotalus atrox*) at Tuzigoot National Monument in Yavapai County, Arizona from April 2022 to September 2022. The snakes were opportunistically treated no more than once a week with either harassment and short distance translocation (“treatment”) or short distance translocation without harassment (“control”). Post-treatment flight distances did not include the distance the snakes were translocated. Blue indicates the treatment group (“+ harassment”) and orange indicates the control group. The graphs include standard error bars.

FIGURE 3-8. Daily mean body temperatures for five adult male Western Diamond-backed Rattlesnakes (*Crotalus atrox*) telemetered at Tuzigoot National Monument in Yavapai County, Arizona from April 2022 to September 2022. The snakes were opportunistically treated no more than once a week with either harassment and short distance translocation (“treatment”) or short distance translocation without harassment (“control”). Graph A shows the mean daily body temperatures of snakes that were in the treatment group including “Gordon” (royal blue line), “Scout” (turquoise line), and “Ted” (navy blue line). Graph B shows the mean daily body temperatures of snakes that were in the control group including “Steve” (red line) and “Jim” (dark red line).

FIGURE 3-9. Daily mean body temperatures for five adult male Western Diamond-backed Rattlesnakes (*Crotalus atrox*) telemetered at Tuzigoot National Monument in Yavapai County, Arizona from April 2022 to September 2022. The snakes were opportunistically treated no more than once a week with either harassment and short distance translocation (“treatment”) or short distance translocation without harassment (“control”). Study subjects include “Gordon” (solid square), “Jim” (solid triangle), “Scout” (open circle), “Steve” (plus sign), “Ted” (open triangle). Colors indicate treatment group (blue = treatment, orange = control).

FIGURE 3-10. Initial defensive behaviors when located by telemetry exhibited by five adult male Western Diamond-backed Rattlesnakes (*Crotalus atrox*) at Tuzigoot National Monument in Yavapai County, Arizona from April 2022 to September 2022. The snakes were opportunistically treated no more than once a week with either harassment and short distance translocation (“treatment”) or short distance translocation without harassment (“control”). The treatment group is represented in graph “A” and the control group is represented in graph “B”. Defensive behavior was broken into four categories: None, Alert, Avoid, and Defend.

FIGURE 3-11. Initial defensive behavior when located by telemetry exhibited by five adult male Western Diamond-backed Rattlesnakes (*Crotalus atrox*) at Tuzigoot National Monument in Yavapai County, Arizona from April 2022 to September 2022. The snakes were opportunistically treated no more than once a week with either harassment and short distance translocation (“treatment”) or short distance translocation without harassment (“control”). The treatment group for each snake is indicated (“T” = treatment and “C” = control). Defensive behavior was broken into three categories: None (blue), Alert (orange), and Avoid (avoid). The behavioral category “Defend” is not represented on the graph because no snakes exhibited initial behaviors in this category.

FIGURE 3-12. Mean plasma corticosterone (CORT) levels post-harassment by treatment group for five adult male telemetered Western Diamond-backed Rattlesnakes (*Crotalus atrox*) at Tuzigoot National Monument in Yavapai County, Arizona from April 2022 to September 2022. The snakes were opportunistically treated no more than once a week with either harassment and short distance translocation (“treatment”, blue) or short distance translocation without harassment (“control”, orange). Standard error bars are given.

TABLE 3-1

Snake Name	**Hectares**	**Mean**	**Standard Error**
Treatment		19.06	+/- 2.61
Gordon	19.44		
Scout	14.37		
Ted	23.37		
Control		32.60	+/- 2.44
Jim	30.16		
Steve	35.04		

TABLE 3-2

Snake Name	Core Area	Count	Mean (Count)	Standard Error (Count)	Hectares	Mean (Hectares)	Standard Error (Hectares)
Treatment			10	+/-2.65		5.98	+/-0.51
Gordon	95%	14			6.47		
Scout		5			6.51		
Ted		11			4.96		
Control			12.5	+/-2.5		7.91	+/-1.24
Jim	95%	15			9.15		
Steve		10			6.67		

TABLE 3-3

Snake Name	**Average Distance Moved Per Day (m)**	**Standard Error**
Treatment	31.30	+/-3.97
Gordon	53.26	+/-16.64
Scout	29.32	+/-6.40
Ted	26.55	+/-4.42
Control	41.01	+/-6.21
Jim	66.87	+/-13.17
Steve	24.90	+/-5.11

TABLE 3-4

Snake Name	Group	Date of Treatment	24 Hours Post-treatment	Moved?	Distance moved (m)
Gordon	Treatment	5/23/2022	5/24/2022	Y	11.66
		7/9/2022	7/10/2022	Y	52.35
		7/18/2022	7/19/2022	Y	258.07
		7/28/2022	7/29/2022	Y	79.25
		8/2/2022	8/3/2022	Y	97.86
Jim	Control	5/30/2022	5/31/2022	Y	23.09
		6/15/2022	6/16/2022	Y	105.12
		6/30/2022	7/1/2022	Y	185.33
		9/13/2022	9/14/2022	Y	37.12
Scout	Treatment	4/25/2022	4/26/2022	Y	90.82
		6/25/2022	6/26/2022	Y	146.86
		6/30/2022	7/1/2022	Y	171.75
		7/6/2022	7/7/2022	Y	125.32
Steve	Control	5/9/2022	5/10/2022	Y	54.12
		6/6/2022	6/7/2022	Y	104.12
		6/15/2022	6/16/2022	Y	101.24
		6/21/2022	6/22/2022	Y	156.55
		7/18/2022	7/19/2022	Y	51.97
		7/26/2022	7/27/2022	Y	138.03
		8/5/2022	8/6/2022	Y	143.39
		8/8/2022	8/9/2022	Y	46.01
		8/15/2022	8/16/2022	Y	39.12
		9/4/2022	9/5/2022	Y	120.04
Ted	Treatment	5/9/2022	5/10/2022	Y	64.20
		5/23/2022	5/24/2022	N	5.83
		7/7/2022	7/8/2022	Y	64.64
		7/14/2022	7/15/2022	Y	80.78
		8/27/2022	8/28/2022	Y	55.00
		9/6/2022	9/7/2022	Y	165.86

TABLE 3-5

Snake Name	**Mean Body Temperature (°C)**	**Standard Error**	**Mean Body Temperature (°C)**	**Standard Error**
Treatment			23.7	+/- 0.1
Gordon	25.6	+/- 0.1		
Ted	23.4	+/- 0.1		
Scout	22.2	+/- 0.1		
Control			25.2	+/- 0.1
Jim	25.2	+/- 0.1		
Steve	25.2	+/- 0.1		

TABLE 3-6

Snake Name	Body Temperature Range (°C)
Treatment	
Gordon	6.1-37.1
Ted	4.0-36.1
Scout	3.6-37.6
Control	
Jim	8.1-36.7
Steve	7.0-37.6

TABLE 3-7

Model #	Model	AIC	Random Effects	p-value
M1	DB~Treatment+(1|Snake_ID)	104.4	$\sigma^2=0$	0.1300
M2	DB~Body_Temp(1|Snake_ID)	104.9	$\sigma^2=0.0028$	0.2953
M3	DB~Days_LT+(1|Snake_ID)	103.2	$\sigma^2=0$	0.0489
M4	DB~Days_EB+(1|Snake_ID)	106.5	$\sigma^2=0.0146$	0.5715
M5	DB~Ambient_Temp+(1|Snake_ID)	106.9	$\sigma^2=1.975e-15$	0.981
M6	DB~Sub_Temp+(1|Snake_ID)	106.1	$\sigma^2=0.0076$	0.546
M7	DB~Time_Of_Day+(1|Snake_ID)	106.5	$\sigma^2=0$	Afternoon (Intercept): 0.423 Evening: 0.383 LateMorning: 0.258 MidDay: 0.218 Morning: 0.624
M9	DB~Time_Of_Day+(1|Snake_ID)	103.3	$\sigma^2=0$	Days_LT: 0.0703 Treatment: 0.1825

TABLE 3-8

Model #	Model	AIC	Random Effects	p-value		ANOVA	p-value
FullModel1	DB~Treatment+Body_Temp+Days_LT+Days_EB+Ambient_Temp+Sub_Temp+Time_of_Day+(1|Snake_ID)	105.8	$\sigma^2=0$	Treatment: 0.1631 Body_Temp: 0.5320 Days_LT: 0.0151 Days_EB: 0.6275 Ambient_Temp: 0.4594 Sub_Temp: 0.8540	Time_Of_Day: Evening: 0.6579 LateMorning: 0.0974 MidDay: 0.1498 Morning: 0.4042		
FullModel2	DB~Treatment+Body_Temp+Days_LT+Days_EB+Ambient_Temp+Sub_Temp+(1|Snake_ID)	103.4	$\sigma^2=0$	Treatment: 0.1744 Body_Temp: 0.1000 Days_LT: 0.0164 Days_EB: 0.1348	Ambient_Temp: 0.8711 Sub_Temp: 0.2282	FullModel1 vs FullModel2	0.2314

TABLE 3-9

Snake Name	**Post-harassment CORT concentration (ng/mL)**	**Mean CORT concentration (ng/mL)**	**Standard Error**
Treatment		97.15	+/-21.28
Gordon	295.75		
Ted	151.19		
Control		223.47	+/-72.28
Jim	118.42		
Steve	75.87		

FIGURE 3-1

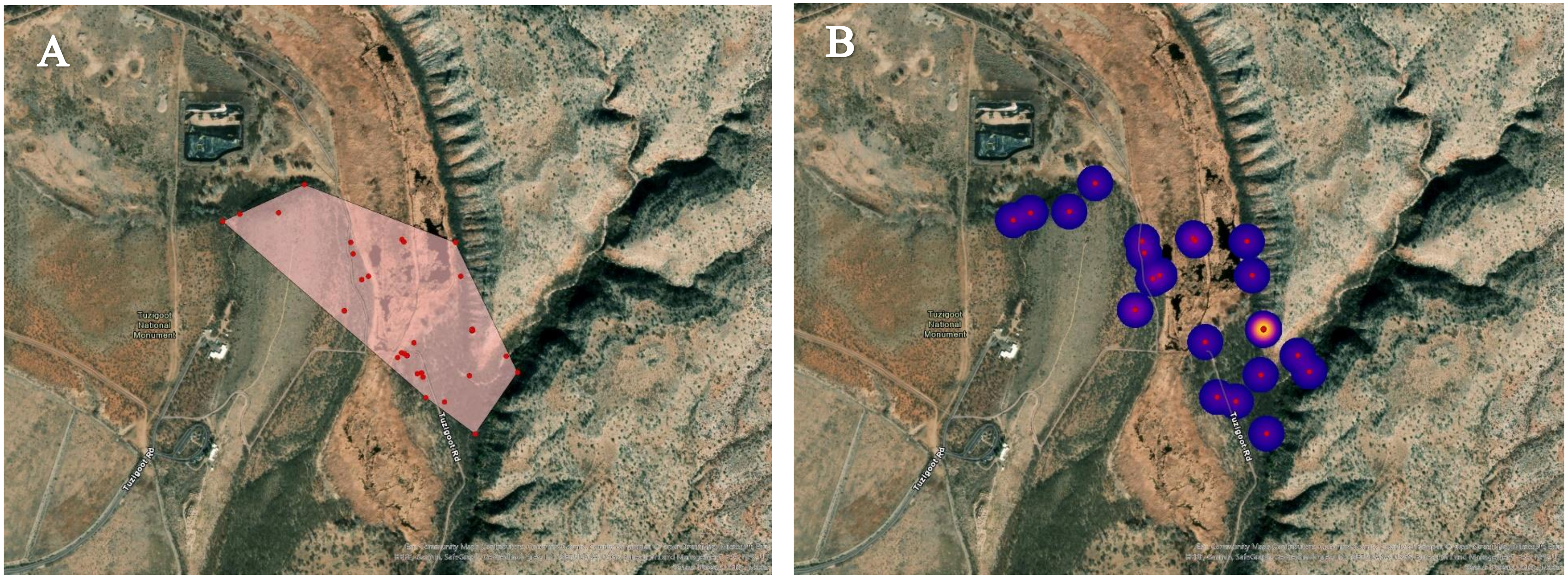

A
Tuzigoot National Monument
Tuzigoot Rd
Tuzigoot Rd
B
Tuzigoot National Monument
Tuzigoot Rd
Tuzigoot Rd

FIGURE 3-2


A
Tuzigoot National Monument
Tuzigoot Rd
Tuzigoot Rd
B
Tuzigoot National Monument
Tuzigoot Rd
Tuzigoot Rd

FIGURE 3-3

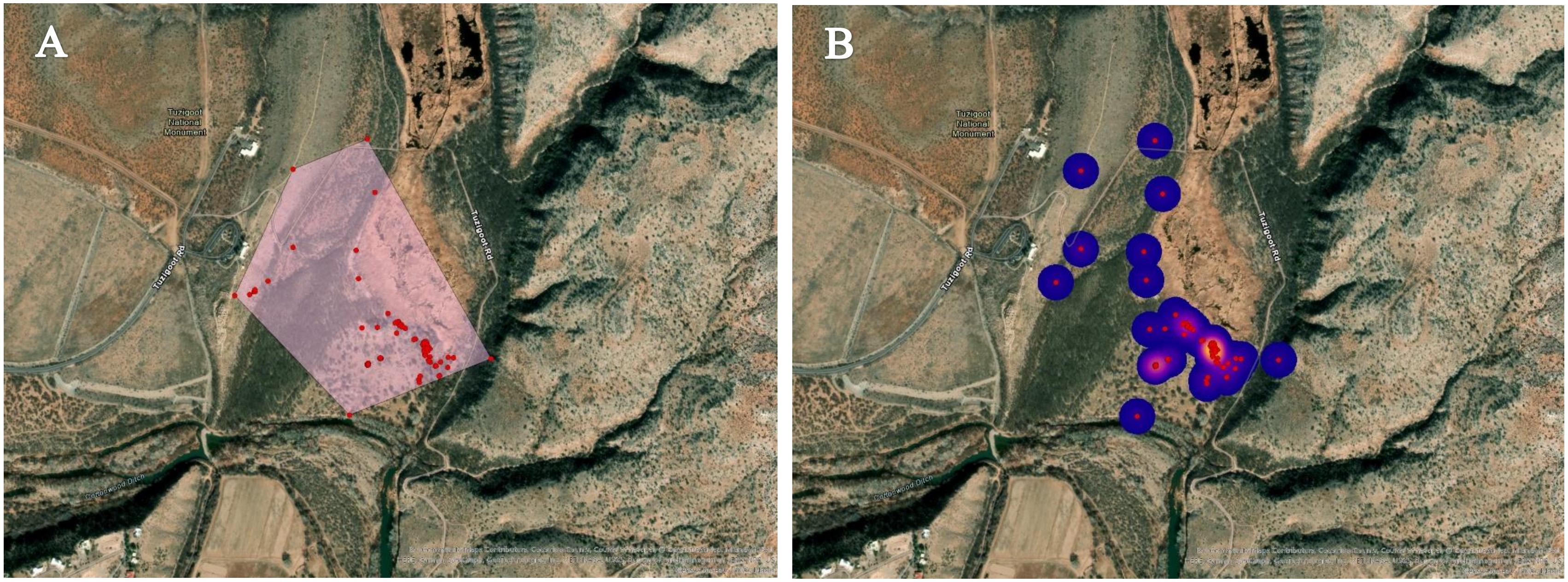

A
B
Tuzigoot National Monument
Tuzigoot Rd
Tuzigoot Rd
Cottonwood Ditch

FIGURE 3-4

FIGURE 3-5

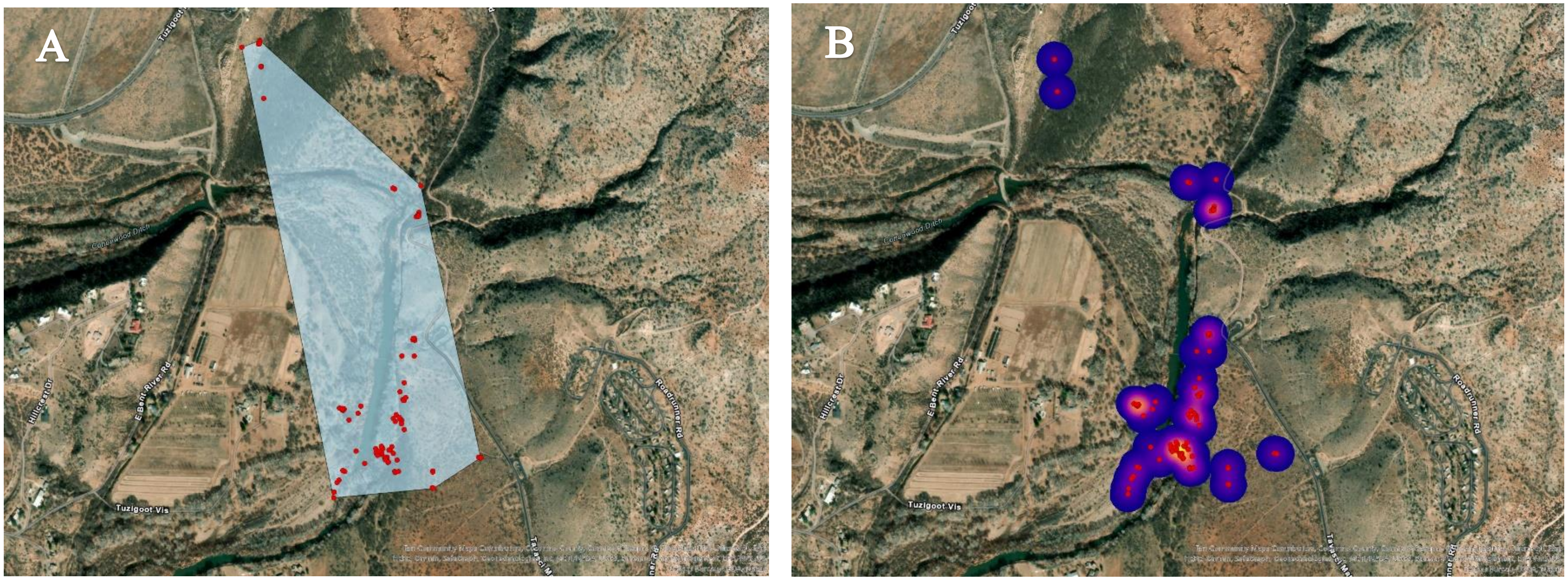

A
B
Tuzigoot
Cottonwood Ditch
Hillcrest Dr
E Bent River Rd
Tuzigoot Vis
Roadrunner Rd

FIGURE 3-6

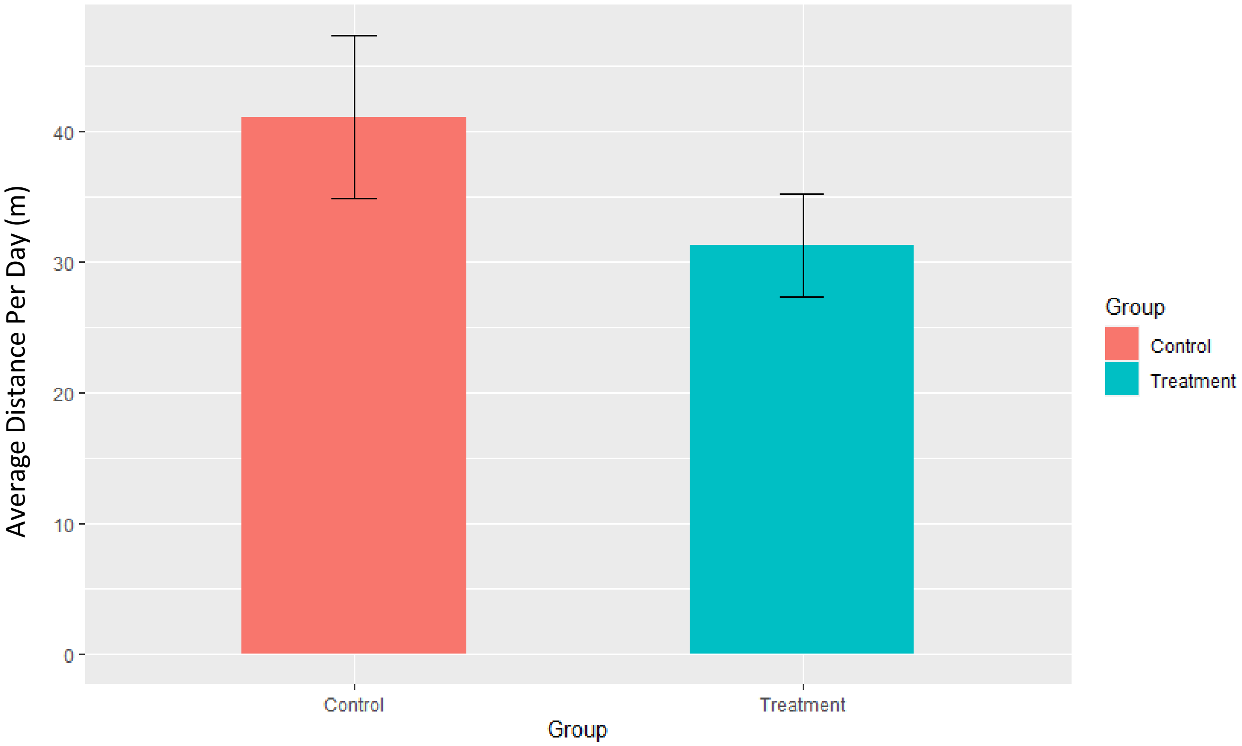

Average Distance Per Day (m)
0
10
20
30
40
Control
Treatment
Group
Group
Control
Treatment

FIGURE 3-7

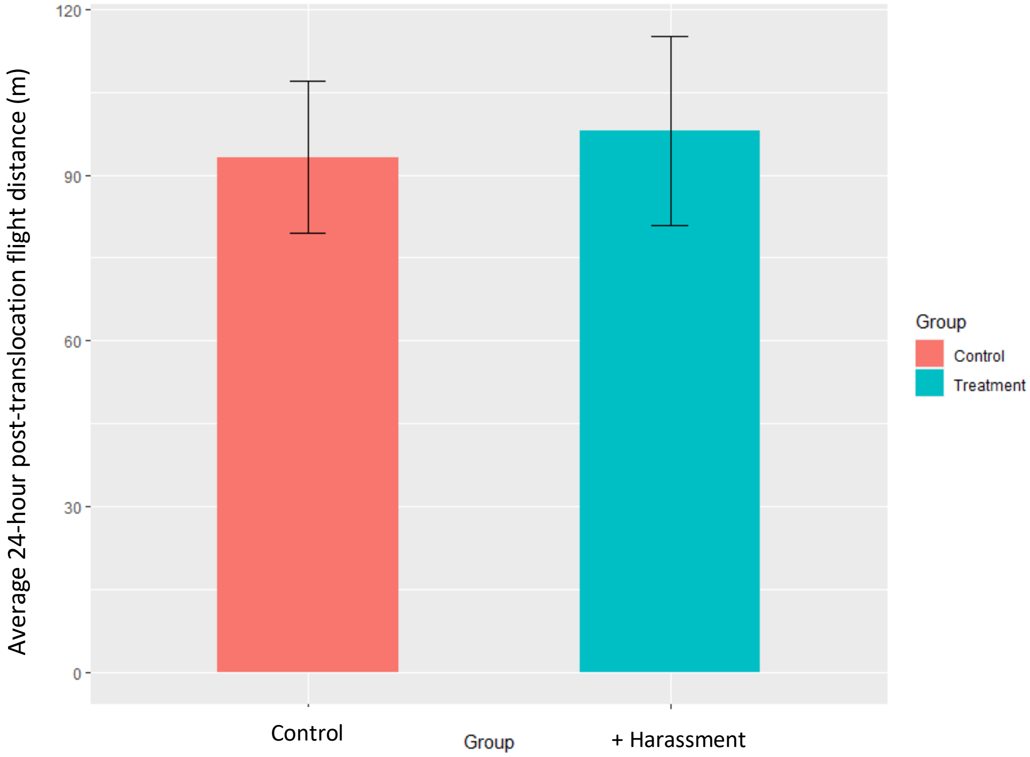

Average 24-hour post-translocation flight distance (m)
120
90
60
30
0
Control
Group
+ Harassment
Group
Control
Treatment

FIGURE 3-8

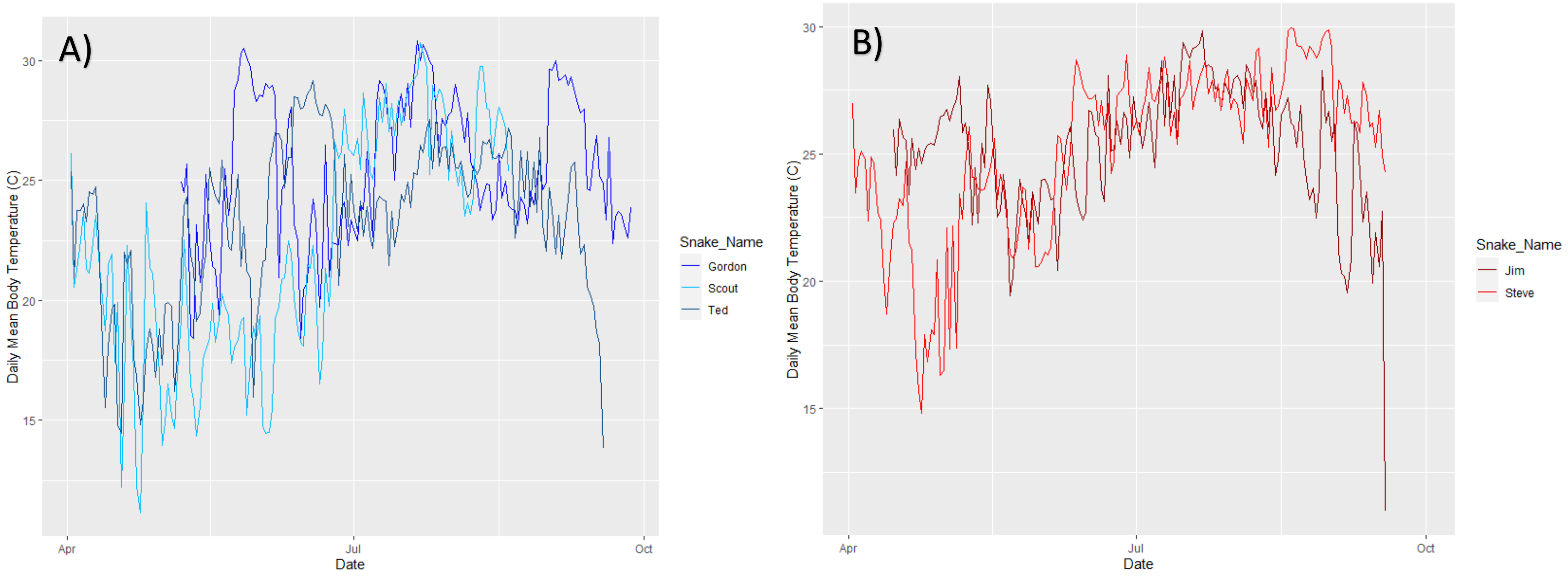

A)
Daily Mean Body Temperature (C)
30
25
20
15
Apr
Jul
Oct
Date
Snake_Name
Gordon
Scout
Ted
B)
Daily Mean Body Temperature (C)
30
25
20
15
Apr
Jul
Oct
Date
Snake_Name
Jim
Steve

FIGURE 3-9

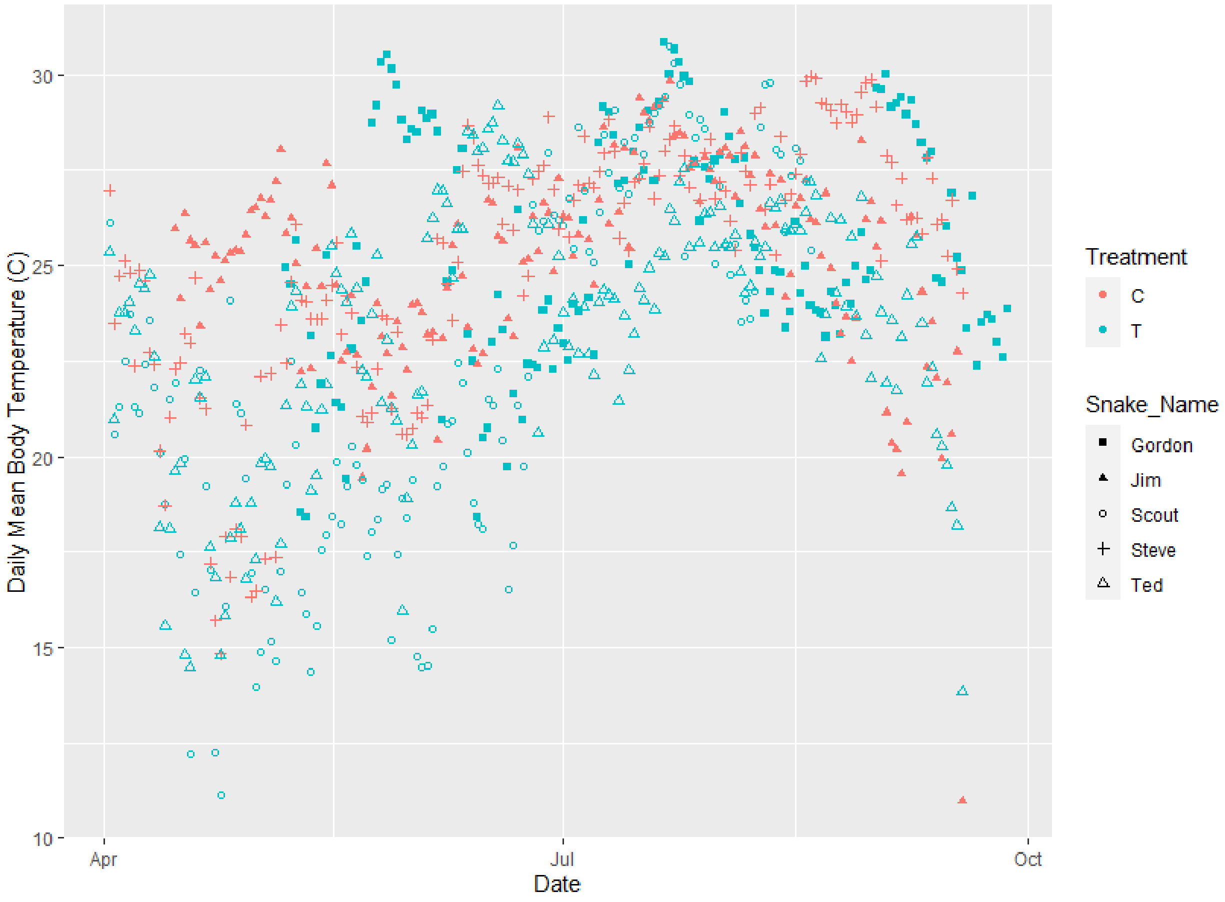

Daily Mean Body Temperature (C)
30
25
20
15
10
Apr
Jul
Oct
Date
Treatment
C
T
Snake_Name
Gordon
Jim
Scout
Steve
Ted

FIGURE 3-10

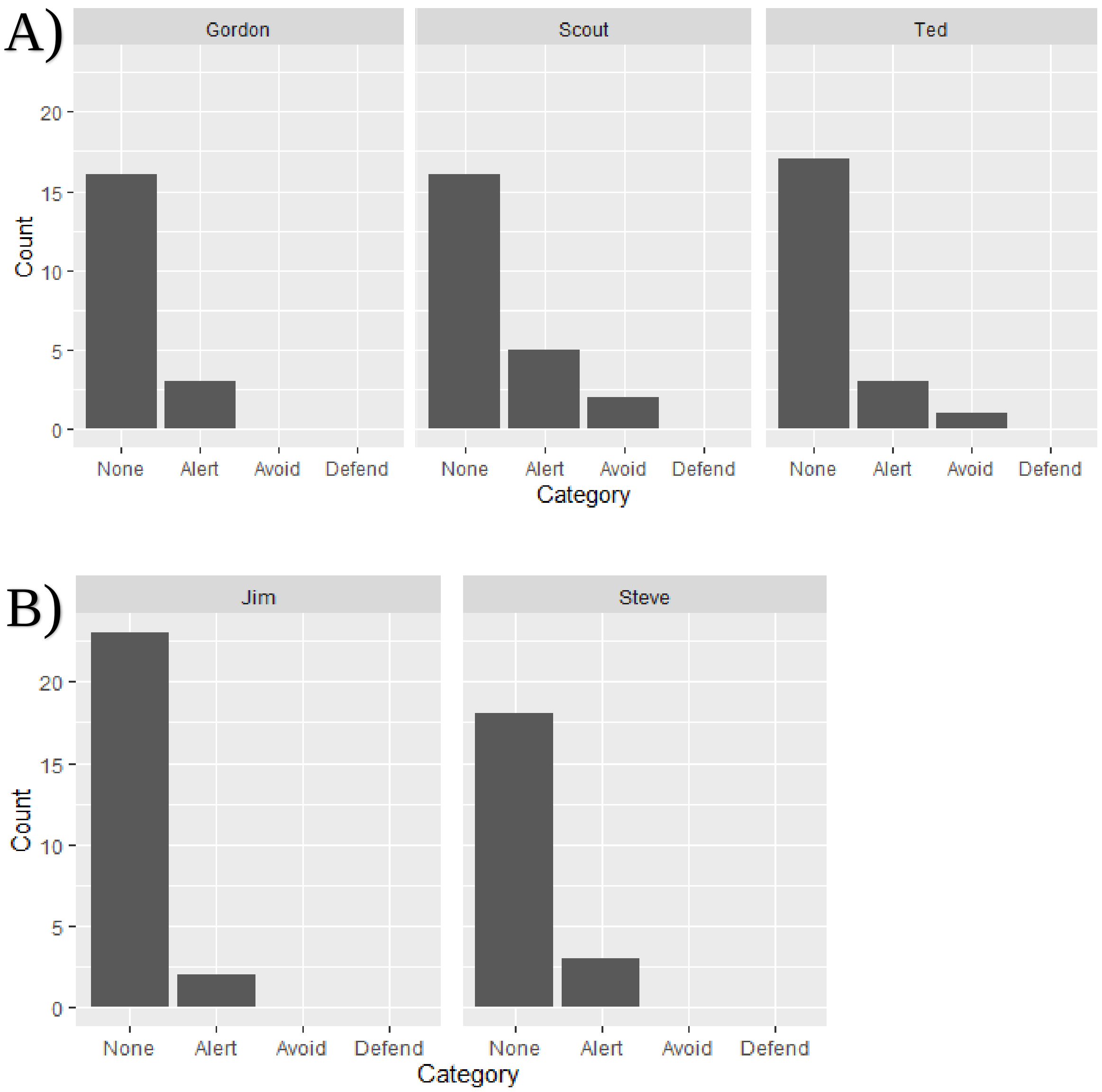
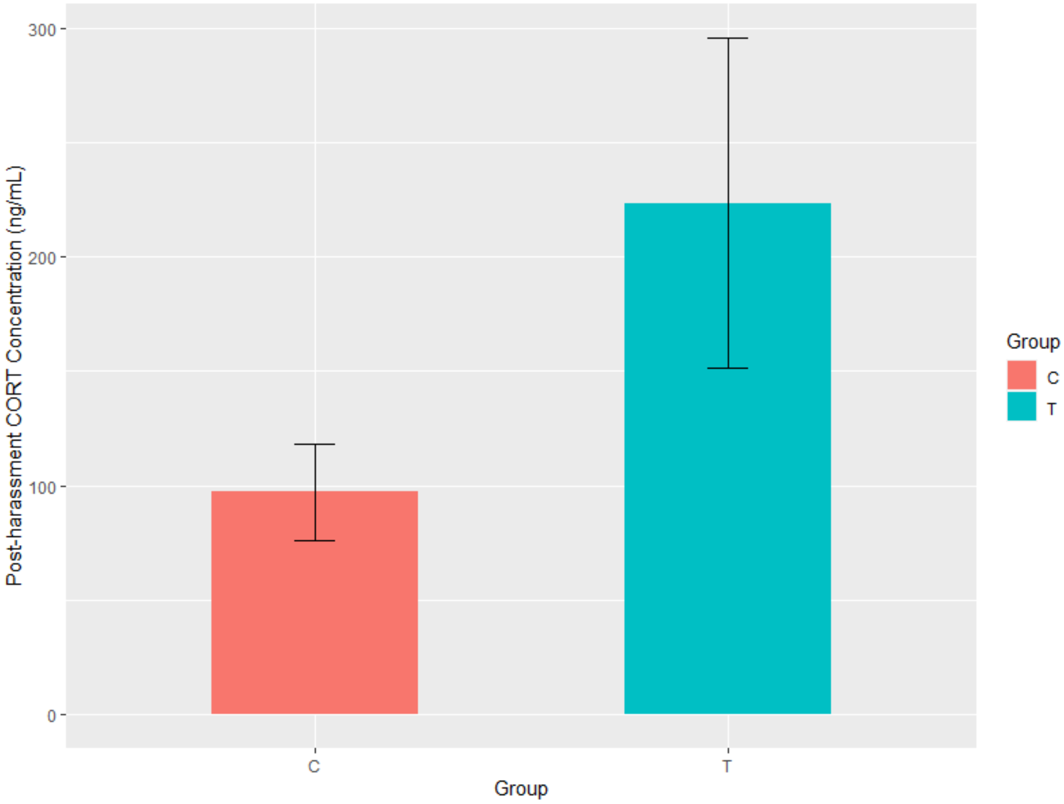

A)
Gordon
Scout
Ted
20
15
10
5
0
Count
None
Alert
Avoid
Defend
Category
B)
Jim
Steve
20
15
10
5
0
Count
None
Alert
Avoid
Defend
Category

FIGURE 3-11


Ted (T)
Steve (C)
Scout (T)
Jim (C)
Gordon (T)
Category
None
Avoid
Alert
Category
Alert
Avoid
None

FIGURE 3-12


300
200
100
0
Post-harassment CORT Concentration (ng/mL)
C
T
Group
Group
C
T

APPENDICES

APPENDIX 1. Daily average movement patterns estimated by A) average 24-hour Overall Dynamic Body Acceleration (ODBA) and B) average 24-hour Vectoral Dynamic Body Acceleration (VeDBA) for the adult male Western Diamond-backed Rattlesnake (*Crotalus atrox*) “Gordon” at Tuzigoot National Monument in Yavapai County, Arizona that was telemetered from April 2022 to September 2022. “Gordon” was opportunistically treated no more than once a week with short distance translocation (SDT) and harassment (“treatment”). Red points indicate days that the snake experienced SDT and harassment.

APPENDIX 2. Daily average movement patterns estimated by A) average 24-hour Overall Dynamic Body Acceleration (ODBA) and B) average 24-hour Vectoral Dynamic Body Acceleration (VeDBA) for the adult male Western Diamond-backed Rattlesnake (*Crotalus atrox*) “Jim” at Tuzigoot National Monument in Yavapai County, Arizona that was telemetered from April 2022 to September 2022. “Jim” was opportunistically treated no more than once a week with short distance translocation (SDT) without harassment (“control”). Red points indicate days that the snake experienced SDT.

APPENDIX 3. Daily average movement patterns estimated by A) average 24-hour Overall Dynamic Body Acceleration (ODBA) and B) average 24-hour Vectoral Dynamic Body Acceleration (VeDBA) for the adult male Western Diamond-backed Rattlesnake (*Crotalus atrox*) “Scout” at Tuzigoot National Monument in Yavapai County, Arizona that was telemetered from April 2022 to September 2022. “Scout” was opportunistically treated no more than once a week with short distance translocation (SDT) and harassment (“treatment”). Red points indicate days that the snake experienced SDT and harassment.

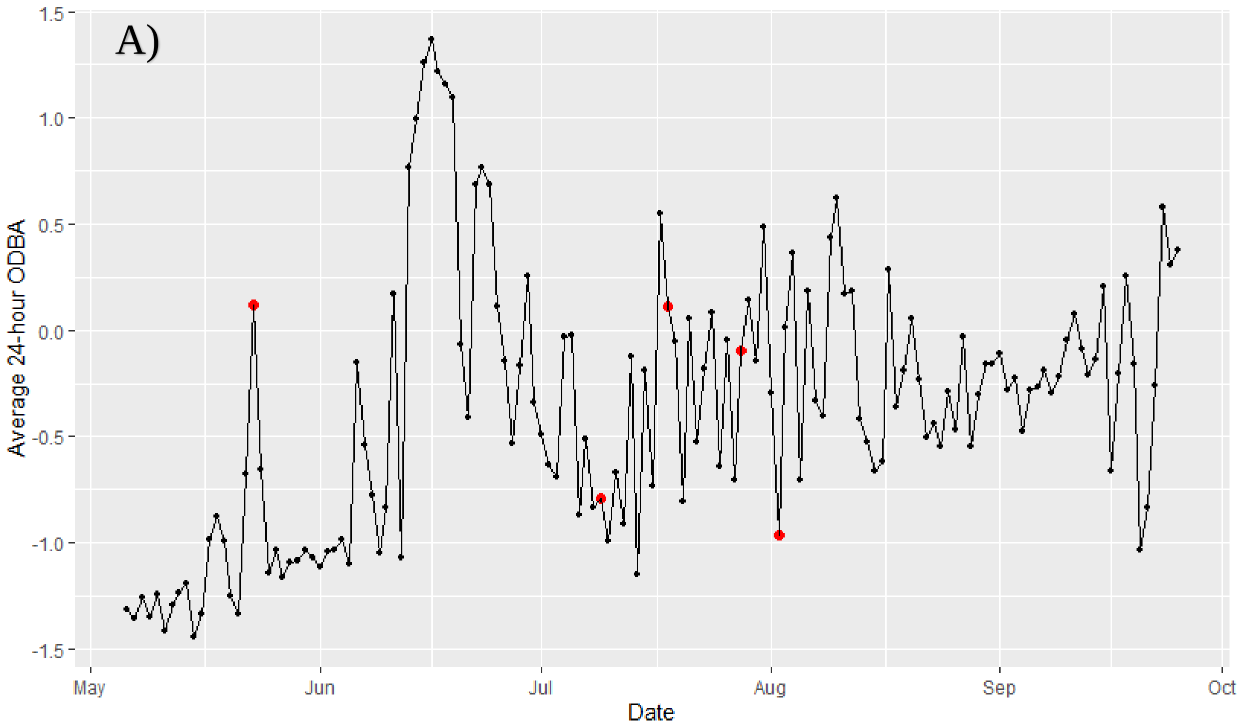
APPENDIX 4. Daily average movement patterns estimated by A) average 24-hour Overall Dynamic Body Acceleration (ODBA) and B) average 24-hour Vectoral Dynamic Body Acceleration (VeDBA) for the adult male Western Diamond-backed Rattlesnake (*Crotalus atrox*) “Ted” at Tuzigoot National Monument in Yavapai County, Arizona that was telemetered from April 2022 to September 2022. “Ted” was opportunistically treated no more than once a week with short distance translocation (SDT) and harassment (“treatment”). Red points indicate days that the snake experienced SDT and harassment.

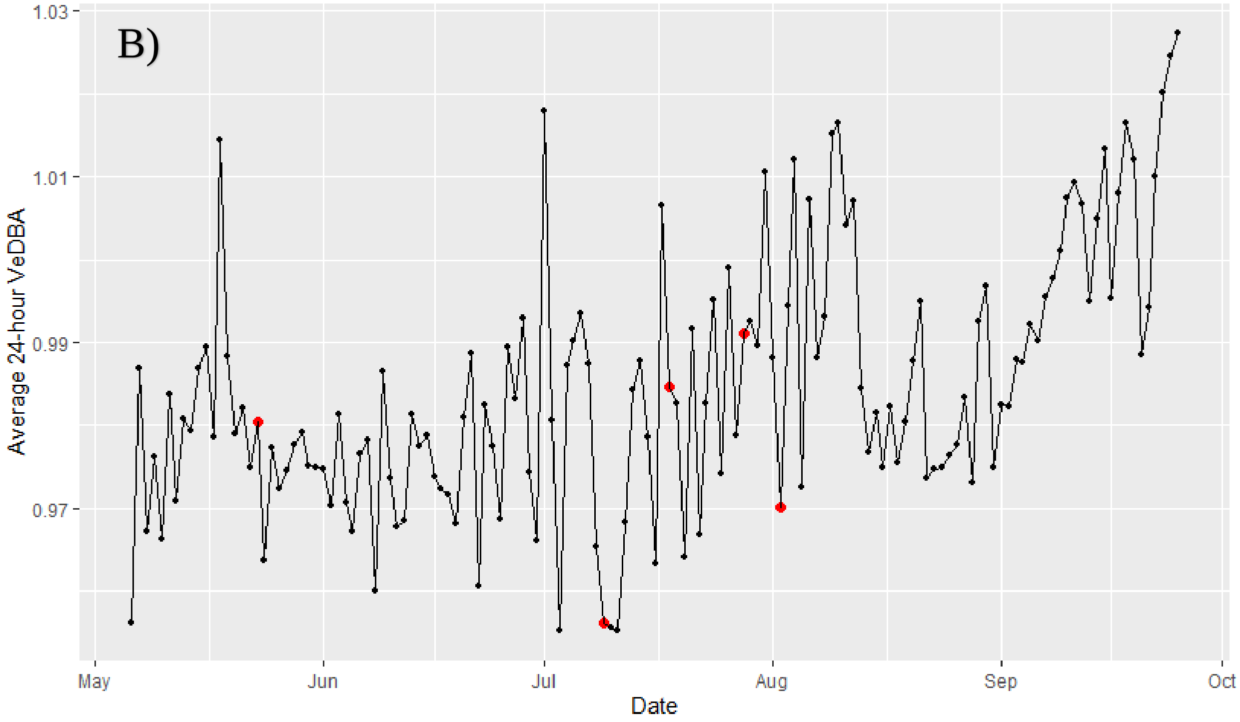
APPENDIX 5. The minimum, mean, and maximum average 24-hour Overall Dynamic Body Acceleration (ODBA) for five adult male Western Diamond-backed Rattlesnakes (*Crotalus atrox*) telemetered at Tuzigoot National Monument, Yavapai County, Arizona from April 2022 to September 2022. The snakes were opportunistically treated no more than once a week with either harassment and short distance translocation (“treatment”) or short distance translocation without harassment (“control”). Higher values are associated with high activity levels, and lower values are associated with low activity levels.

APPENDIX 6. Average 24-hour Overall Dynamic Body Acceleration (ODBA) of five adult male Western Diamond-backed Rattlesnakes (*Crotalus atrox*) telemetered at Tuzigoot National Monument, Yavapai County, Arizona from April 2022 to September 2022. The snakes were opportunistically treated no more than once a week with either harassment and short distance translocation (“treatment”) or short distance translocation without harassment (“control”). “Treatment Day” refers to days that snakes experienced either translocation alone or translocation and harassment. “Y” indicates days that snakes experienced either harassment and short distance translocation or short distance translocation alone, and “N” indicates days that snakes did not experience any experimentation.

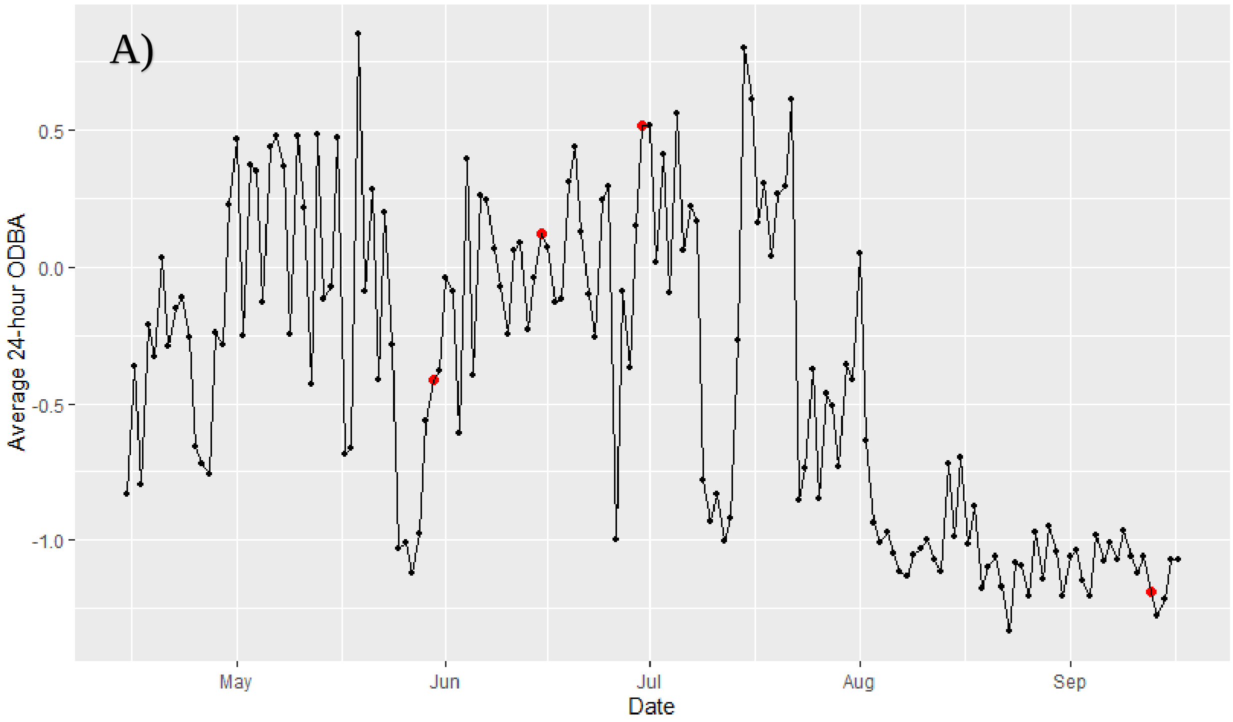
APPENDIX 7. Average 24-hour Overall Dynamic Body Acceleration (ODBA) of five adult male Western Diamond-backed Rattlesnakes (*Crotalus atrox*) telemetered at Tuzigoot National Monument, Yavapai County, Arizona from April 2022 to September 2022. The snakes were opportunistically treated no more than once a week with either harassment and short distance translocation (“treatment”) or short distance translocation without harassment (“control”). The boxplots are separated by 24-hours post-treatment day: “Y” indicates days that were 24-hours after the snakes experienced either harassment and short distance translocation or short distance translocation alone, and “N” indicates all other days.

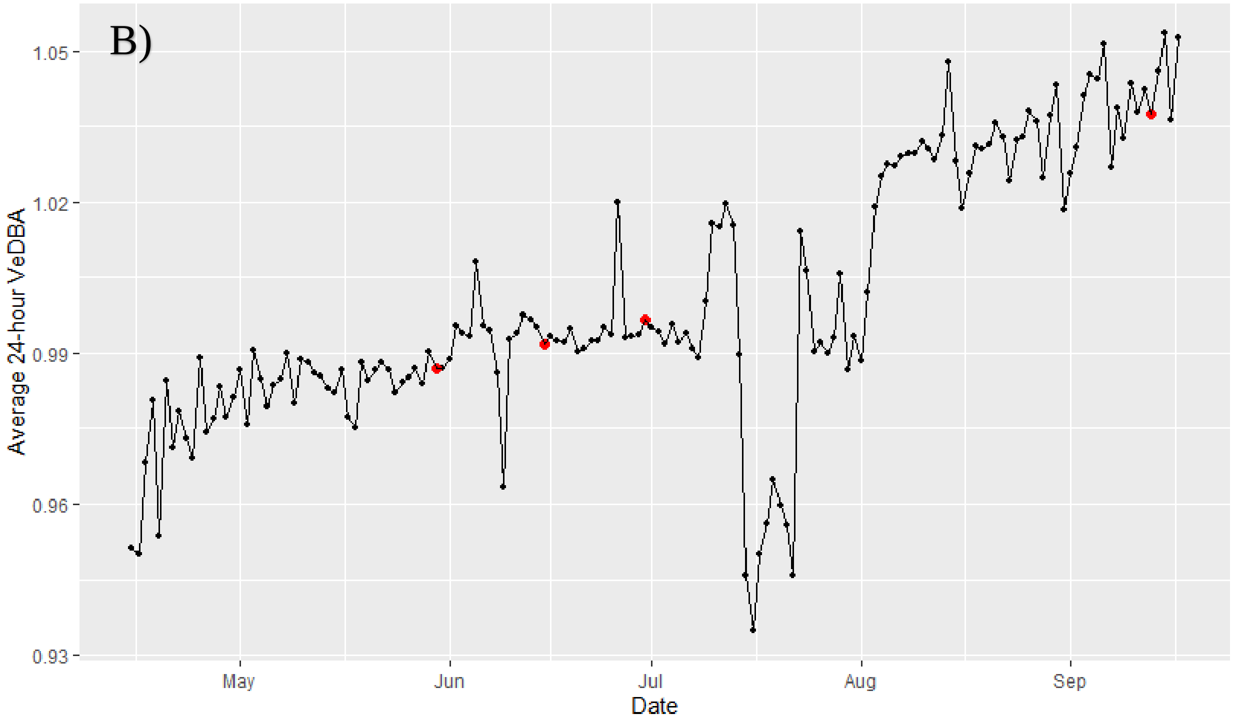
APPENDIX 1.


A)
Average 24-hour ODBA
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
May
Jun
Jul
Aug
Sep
Oct
Date

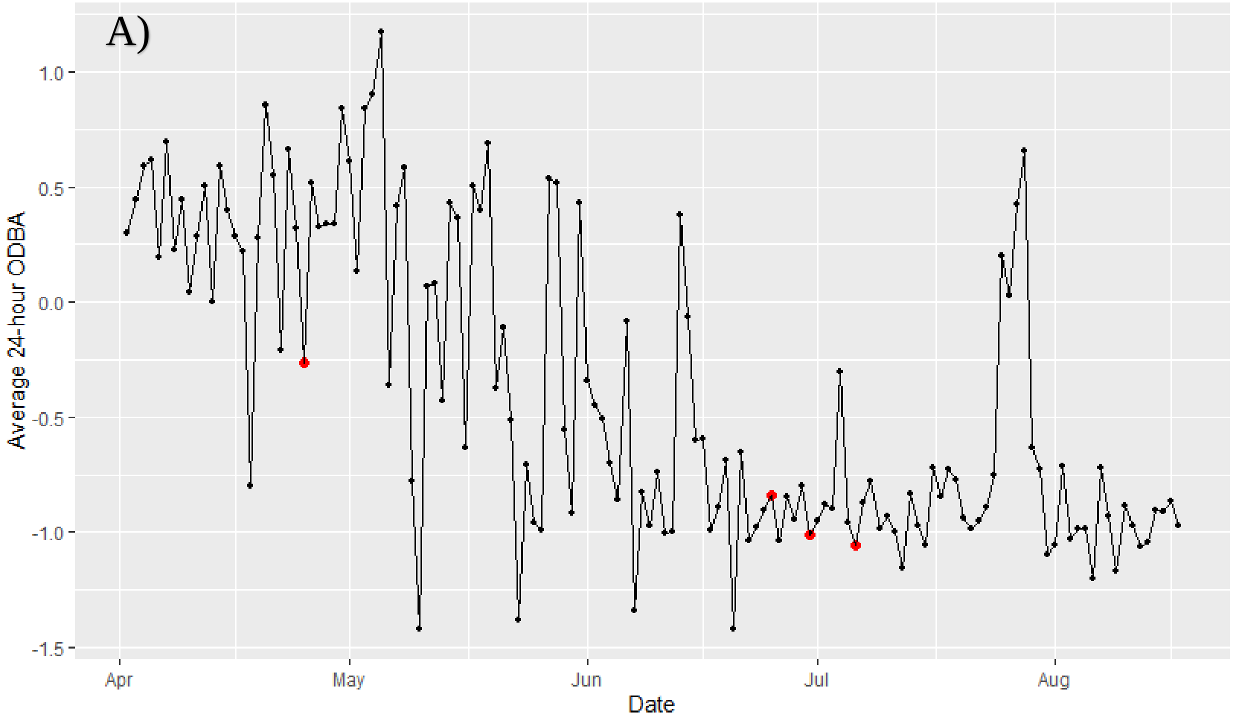

B)
Average 24-hour VeDBA
1.03
1.01
0.99
0.97
May
Jun
Jul
Aug
Sep
Oct
Date

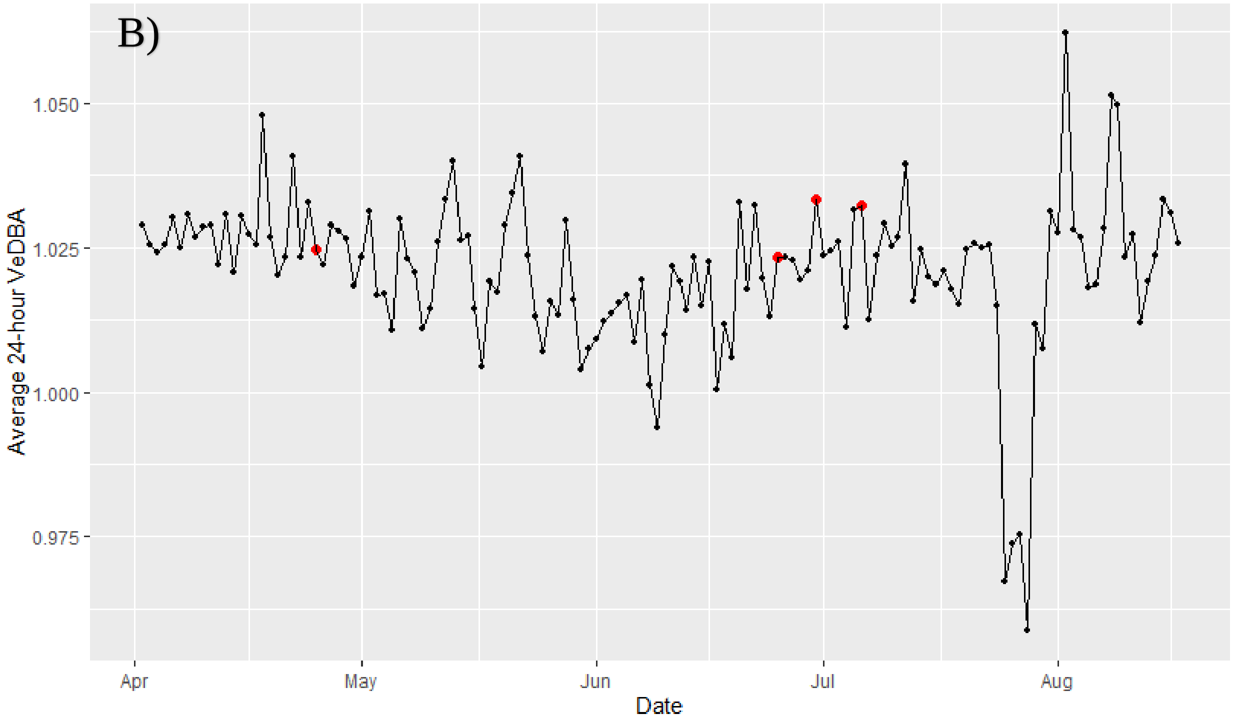
APPENDIX 2.


A)
Average 24-hour ODBA
0.5
0.0
-0.5
-1.0
May
Jun
Jul
Aug
Sep
Date

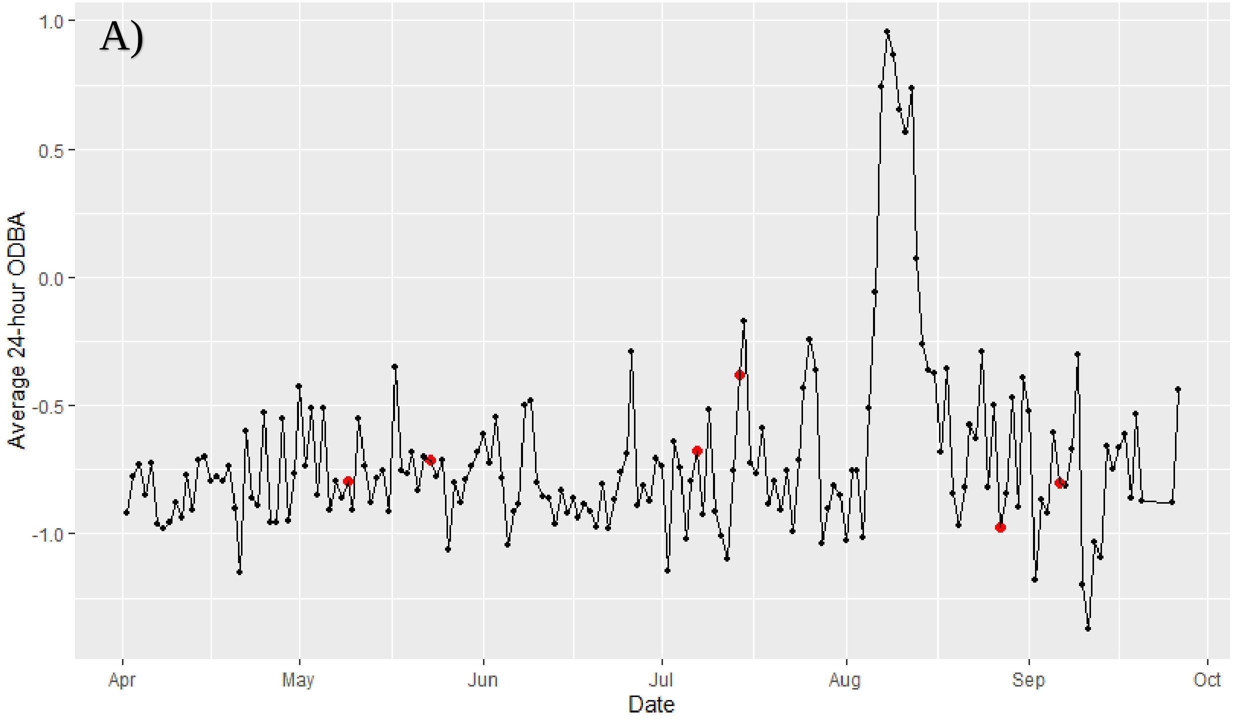

B)
Average 24-hour VeDBA
1.05
1.02
0.99
0.96
0.93
May
Jun
Jul
Aug
Sep
Date

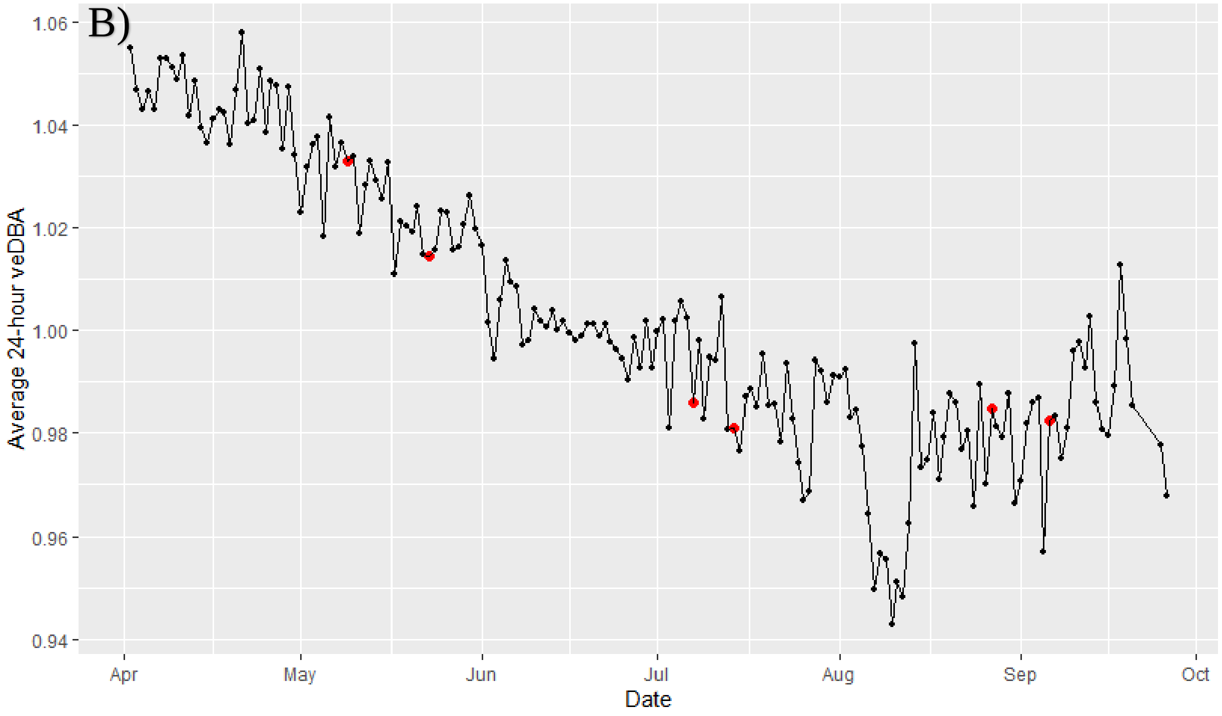
APPENDIX 3.


A)
Average 24-hour ODBA
1.0
0.5
0.0
-0.5
-1.0
-1.5
Apr
May
Jun
Jul
Aug
Date


B)
Average 24-hour VeDBA
1.050
1.025
1.000
0.975
Apr
May
Jun
Jul
Aug
Date

APPENDIX 4.

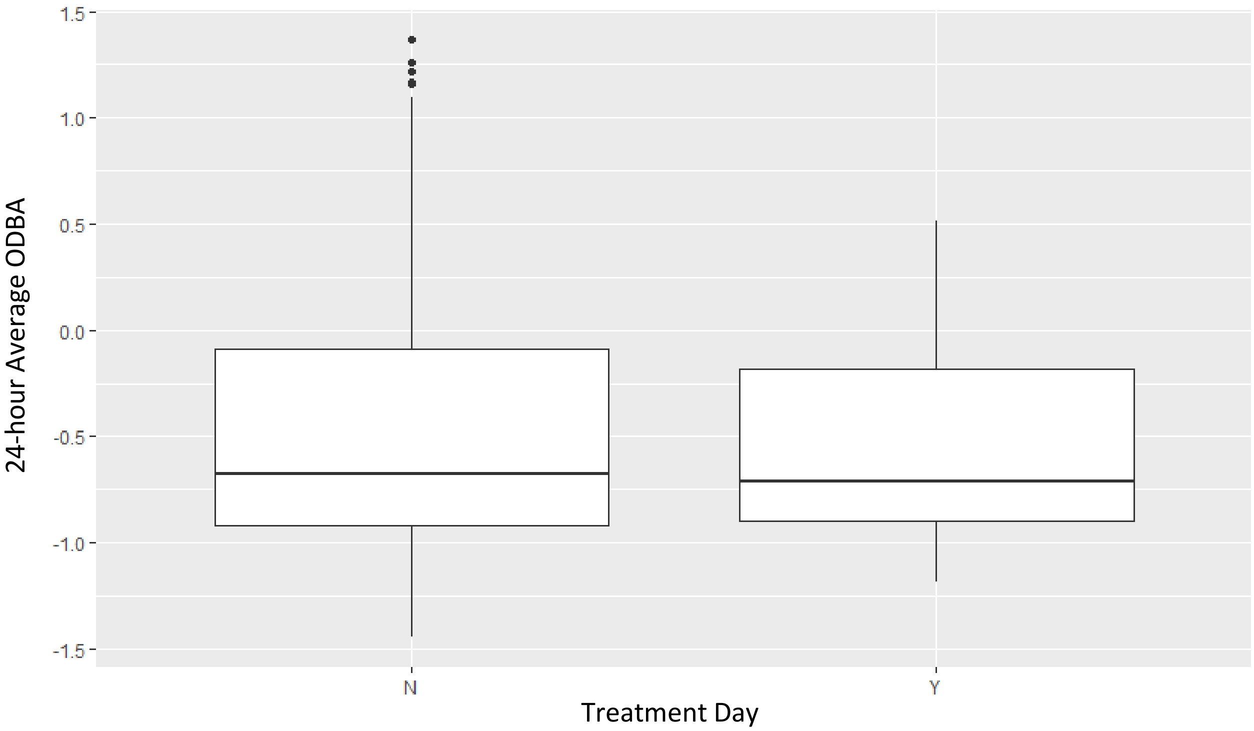

A)
1.0
0.5
0.0
-0.5
-1.0
Average 24-hour ODBA
Apr
May
Jun
Jul
Aug
Sep
Oct
Date


B)
1.06
1.04
1.02
1.00
0.98
0.96
0.94
Average 24-hour veDBA
Apr
May
Jun
Jul
Aug
Sep
Oct
Date

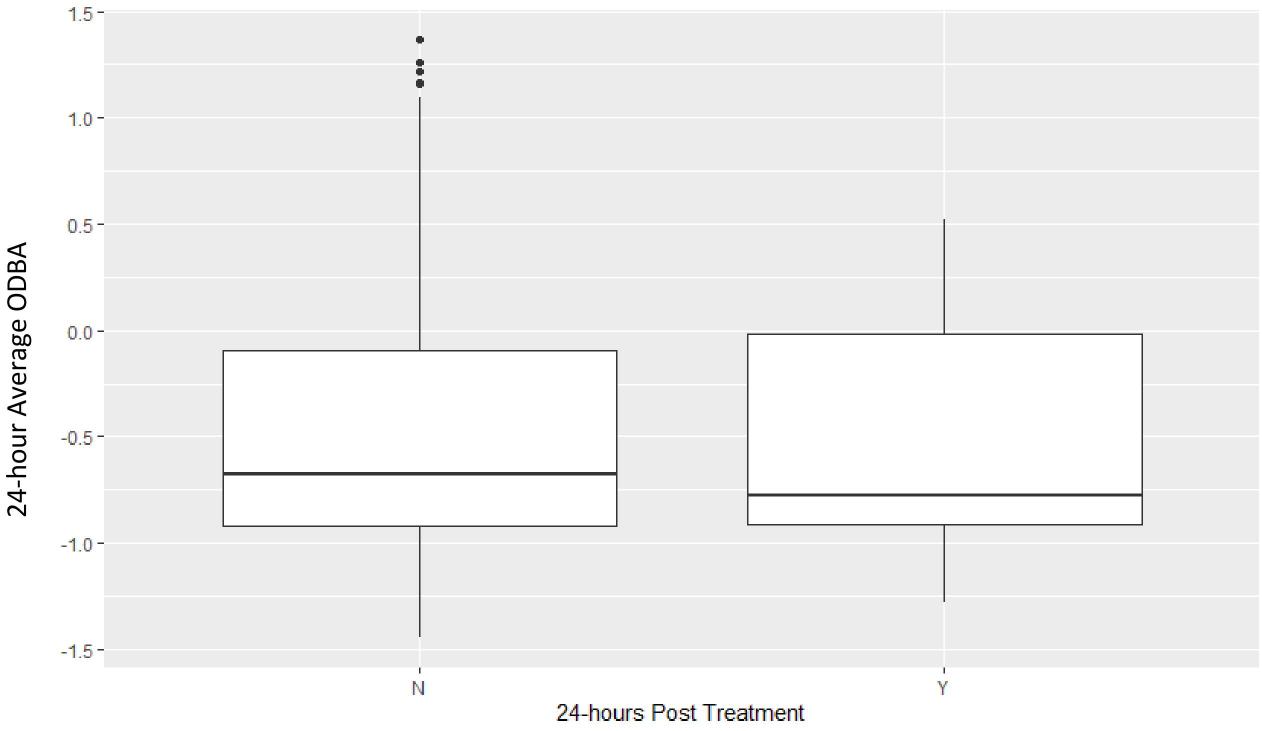
APPENDIX 5.

Snake Name	**Minimum 24-hour ODBA**	**Mean 24-hour ODBA**	**Maximum 24-Hour ODBA**
Treatment			
Gordon	-1.4471	-0.3328	1.3654
Ted	-1.3731	-0.7050	0.9530
Scout	-1.4228	-0.3864	1.1693
Control			
Jim	-1.3336	-0.4115	0.8473

APPENDIX 6.



APPENDIX 7.


1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
24-hour Average ODBA
N
Y
24-hours Post Treatment