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# Movement Ecology Of A Cryptic Ambush Predator: Integrating Radio Telemetry And Tri-Axial Accelerometry To Evaluate Spatial Strategies And Activity Patterns By Western Diamond-Backed Rattlesnakes (*Crotalus atrox*)

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MOVEMENT ECOLOGY OF A CRYPTIC AMBUSH PREDATOR: INTEGRATING RADIO  
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STRATEGIES AND ACTIVITY PATTERNS BY WESTERN  
DIAMOND-BACKED RATTLESNAKES (*Crotalus atrox*)

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Stephen Crites, Ph.D.  
Dean of the Graduate School

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## **Dedication**

*For Lindsey and Shaylee*



MOVEMENT ECOLOGY OF A CRYPTIC AMBUSH PREDATOR: INTEGRATING RADIO  
TELEMETRY AND TRI-AXIAL ACCELEROMETRY TO EVALUATE SPATIAL  
STRATEGIES AND ACTIVITY PATTERNS BY WESTERN  
DIAMOND-BACKED RATTLESNAKES (*Crotalus atrox*)

by

DOMINIC LOUIS DESANTIS, B.S.

DISSERTATION

Presented to the Faculty of the Graduate School of  
The University of Texas at El Paso  
in Partial Fulfillment  
of the Requirements  
for the Degree of

DOCTOR OF PHILOSOPHY

Department of Biological Sciences  
THE UNIVERSITY OF TEXAS AT EL PASO

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*“Happiness only real when shared”* – Christopher McCandless

Managing the many professional responsibilities that accompany the pursuit of a doctoral degree can be an overwhelming endeavor, at times, but also a tremendously fulfilling accomplishment. However, for myself, overcoming that challenge while allotting the appropriate care and attention to personal responsibilities was a foolishly underappreciated ingredient in the recipe for a successful graduate experience. Upon reflection, I can humbly admit to initially failing to strike this balance. Fortunately, it is only through failure that we can learn to fully appreciate our achievements, and this perspective guided me through the latter portions of my Ph.D. The above quote from McCandless is a core principle that I adopted as an adolescent, lost sight of, and regained in the completion of my dissertation. Without the help and support of numerous colleagues, friends, and family, this work and all of the joy experienced and shared along the way would not have been realized.

I have dedicated this dissertation to my wife, Lindsey, and our daughter, Shaylee. Lindsey, I’ve never known a better feeling than returning home to your kindness and optimism after my MANY long days in the field or office. I would be remiss to not thank you for carrying and delivering our angelic daughter, Shaylee, as there are truly no words to adequately convey my admiration and gratitude in this regard. She will be approaching her first birthday at the time of my graduation, and it is and will continue to be, my greatest privilege to raise her with you. I also thank my parents, Lou and Renee, who never balked at encouraging me to pursue my dreams, no matter how “creepy-crawly” they were. I thank my sister, Gabrielle, who has never hesitated to

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## Abstract

An animal's decision to move from one location to another within its environment is determined by a complex blend of internal and external factors. Teasing apart the relative roles of specific variables in this web of interacting mechanisms has been a long-standing challenge in animal movement ecology. Historically, this problem was viewed as a sort of black box for which a myriad of methodological limitations precluded rigorous study. Recently, a diversity of animal-borne transmitters and dataloggers (i.e., bio-loggers) have circumvented many of these traditional limitations and transformed field studies of animal movement, behavior, and physiology – in some cases, allowing for testing of entirely new theories. Among these technologies, tri-axial accelerometers (ACTs), which enable remote and continuous recording of animal activity, are becoming increasingly commonplace in longitudinal field studies. One of the many strengths offered by ACTs is the ability to be paired with other sensors to provide multiple, complementary data types. In the recent explosion of such integrative bio-logging applications, a distinct taxonomic bias is evident, with smaller-bodied terrestrial taxa often being overlooked because of greater difficulty in device attachment or implantation. Significant computational challenges also remain with these “big data” that are often exacerbated in pilot studies with novel study species. Herein, an integrative framework coupling radio telemetry and accelerometry (RT-ACT) is developed and validated through a case study on Western Diamond-backed Rattlesnakes (*Crotalus atrox*), representing the first example of snakes as a focal organism in bio-logging research. Telemetry proved to be critical in ACT validation procedures, enabling periodic field observations of rattlesnake behavior that were used to train and test supervised machine learning models for behavioral classification. Following model training, Random Forest and Generalized Linear-NET algorithms distinguished between periods of “activity” and “inactivity” at very high accuracies

(99.0% and 97.0%, receptively), allowing automated classification of activity in extensive ACT field datasets ( $94 \pm 99$  days, range = 6–289 days). These classifications enabled the construction of continuous activity budgets for evaluation of the timing and duration of activity at multiple temporal scales. In general, activity patterns were found to be highly variable within and between individuals, as the proportion of time spent “active” per individual dataset ranged from 1.6% to 37.1%. The same general daily activity pattern was conserved across all active seasons (spring, summer, fall), with the majority of activity occurring during the evening or nocturnal diel periods. There was seasonal variation in activity duration within diel periods, as activity increased during the summer-mating season, possibly reflective of characteristic mate-searching efforts by male rattlesnakes. Moving forward, long-term and low-frequency ACT field-monitoring could play an important role in improving our understanding of organismal responses to shifting environmental conditions, particularly in small, secretive terrestrial taxa for which other bio-logging technologies are not applicable. Independent captive observations might also allow classification of additional cryptic behaviors (even at very low ACT recording frequencies (1-Hz)) not often observed in the field, ultimately enabling real-time tracking of individual behavior and performance that can be linked to population dynamics.

In addition to its role in validating the RT-ACT framework, radio telemetry was also used to explore the effects of sex, behavioral season, and critical resource distribution on the spatial strategies of *C. atrox*. Specifically, seasonal movement and space use patterns were used to test whether Native Habitats (NH) and human-made Resource Hotspots (RH) on the Indio Mountains Research Station facilitate divergent search strategies in response to critical resources, including potential mating partners, being widely dispersed in NH and clustered in RH. Independent of habitat category, seasonal patterns largely reflected those expected in a male-search based mating

system. However, accounting for individuals using primarily NH and those using RH revealed divergent strategies. NH males used more space than RH males within both behavioral seasons, and NH males increased movement distances and space use during the mating season while RH males displayed no significant seasonal shifts. NH females elevated movement distances during the mating season while RH displayed no seasonal shifts in movement or space use. Collectively, seasonal spatial patterns and observations of reproductive behavior uncovered contrasting patterns by NH and RH individuals that might represent alternative optimal strategies in this unique system, highlighting the potential for multiple interacting mechanisms (sexual selection, behavioral plasticity, and habitat heterogeneity) to drive disparate tactics within populations. In summary, this dissertation illustrates: 1) the transformative potential of integrative bio-logging approaches in field studies of movement behavior through the development and validation of the novel RT-ACT framework, and 2) the retained value of traditional data collection techniques (i.e., radio telemetry) in specific contexts.

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## **Note to Readers**

I have divided this dissertation into four primary chapters: Chapter 1 is a general introduction that outlines the motivation for research presented in Chapters 2 and 3. Chapter 2 serves as a validation study for a novel integrative methodological framework with applied extensions of the technique therein. Chapter 3 is an in-depth evaluation of multiple interacting mechanisms that combine to drive variation in spatial strategies. Chapter 4 is a conclusion section that summarizes the findings from Chapters 2 and 3 with commentary on the broader implications of these results and directions for future research. This layout results in some occasional overlap of introductory content between chapters. Citations for all sections are compiled into a single reference list at the end of the dissertation. Chapters 1 and 4 are written in the first-person singular, as they were compiled entirely by myself, while Chapters 2 and 3 are written in the first-person plural, given that they were collaborative efforts – in the case of these projects, I conducted the majority of the work presented and I am the lead author for all manuscripts submitted for publication produced from those chapters.

## Chapter 1: Introduction

Animal movement is a fundamental ecological process that plays a critical role in the fitness and evolution of species (Nathan et al. 2008). Humans have long been fascinated by movement, illustrated by Aristotle writing on the motion of animals over 2000 years ago in his text, *De Motu Animalium* (Nussbaum 1978). It is therefore unsurprising that the scientific study of animal movement has a deep history (Hooten et al. 2017), and, like many other fields, great leaps forward have often corresponded with technological innovations leading to transformative methods (Wilmers et al. 2015; Allan et al. 2018). Studying the behavior of wild animals presents many challenges, particularly for species that are elusive and inhabit remote or inaccessible terrain. These challenges, coupled with the broader importance of animal movement in ecosystem structure and function, likely motivated the Craighead brothers to develop and deploy the first radio telemetry collars on Grizzly Bears (*Ursus arctos*) in the 1960s (Craighead and Craighead 1972; Hooten et al. 2017). Today, advanced animal telemetry and data-logging devices have revolutionized the field of animal movement ecology. Satellite collars can provide massive quantities of position data over long time scales, remote sensing technologies offer ever-improving data on environmental conditions, and animal-borne remote-sensing, or bio-logging, devices have given researchers the ability to monitor physiology and behavior at resolutions and precisions historically restricted to laboratories (Ropert-Coudert and Wilson 2005; Kays et al. 2015; Allan et al. 2018). A recent shift in the way scientists approach the study of animal movement has also contributed to this methodological revolution, as, historically, researchers were interested in the redistribution of populations through time (i.e., the Eulerian approach), whereas current workers are more focused on quantifying the movement of individual organisms

(i.e., the Lagrangian approach) (Nathan et al. 2008). Traditionally, the Lagrangian approach was applied to the movement of microorganisms, but the recent and rapid improvements in animal-borne technologies spurred significant progress in applications with large vertebrates, to the point where such examples are now commonplace (Brown et al. 2013; Allan et al. 2018). An emerging frontier in animal movement ecology is to now capitalize on recent and ongoing advancements and miniaturization in these technologies and widen the breadth of taxa available to such applications.

Animal-borne bio-logging techniques are not new to ecology, but rather the complexity, multidimensionality, and interconnectedness of sensors are largely responsible for the recent popularity of “techno-ecological” frameworks (Allan et al. 2018). “Bio-logging” refers to the use of a variety of animal-borne tags or sensors that record data related to an individual animal’s movement, behavior, physiology, and/or environment (Wilmers et al. 2015). Among the many bio-logging sensors available to ecologists, the use of miniature tri-axial accelerometers is becoming increasingly prevalent (Halsey et al. 2009; Shepard et al. 2010; Brown et al. 2013; Wilmers et al. 2015). Accelerometers (ACTs) allow remote monitoring of animal activity free from limitations of observer presence, terrain, weather, time of day, and scale of space use (Yoda et al. 1999; Wilson et al. 2006; Brown et al. 2013). ACTs function as small (often < 1 g) piezoelectric (spring-like) sensors that measure “dynamic” acceleration derived from subject motion, superimposed upon “static” acceleration from gravity (Yoda et al. 1999; Brown et al. 2013). When the device is not in motion, or when an animal is at rest, the only force acting on the device is gravitational acceleration, allowing for accurate identification of the timing, duration, and intensity of movement-mediated behavior. Both static and dynamic acceleration can be interpreted independently to inform understanding of animal orientation (posture) and movement,

respectively, allowing insights into a wide range of behaviors (Shepard et al. 2008). ACTs have effectively revolutionized field studies of wild animal behavior and eco-physiology, allowing for the development and testing of entirely new theories in animal movement and behavioral ecology (Wilmers et al. 2015; Westley et al. 2018).

Despite the transformative potential of these technologies, considerable limitations remain with most animal-attached sensors. The most prominent of these limitations include the overall size of devices (and associated tag-effects on animals), the trade-offs between battery power, sampling frequency, and recording duration, and a lack of standardization in collection, processing, and analysis procedures, with exceedingly large and computationally demanding datasets (Brown et al. 2013; Collins et al. 2015; Allan et al. 2018; Wilson et al. 2019). These constraints are exacerbated in pilot studies with novel focal organisms, and particularly those involving small, secretive, and cryptic species for which *a priori* knowledge of behavior is meager (Reshef et al. 2014; Chimienti et al. 2016; Hammond et al. 2016). An additional barrier to realizing the full potential of ACTs in applied contexts is the general difficulty in recording behavior over significant periods in the field, as relatively short-term deployments (with small sample sizes) often constrain the ability to draw biological or ecological conclusions from ACT data. Most studies make use of a high monitoring frequency ( $\geq 25$  Hz) to improve accuracy in classification of multiple distinct behaviors, but severely limit field recording duration by doing so. However, similar model accuracy can sometimes be achieved even after significant down-sampling (e.g., 25 to 10-Hz in Wang et al. 2015; 64 to 8-Hz in Alvarenga et al. 2016), and it was recently demonstrated that distinct behavioral modes can be accurately classified from ACT data recorded at very low sampling frequencies (i.e., 1-Hz) (Tatler et al. 2018). When coupled with continued improvements in batteries and logger memory capacity, extensive field deployments



with continuous data-logging are now attainable. Although it should be noted that Tatler et al. (2018) validated their approach via captive observation experiments with Dingoes (*Canis dingo*), therefore, the efficacy of long-term deployments at low ACT monitoring frequencies remains to be tested in the field and with other study species and systems. Further, although ongoing miniaturization of ACT dataloggers and associated batteries is expanding the diversity of organisms amenable to external attachment or internal implantation techniques (Brown et al. 2013; Tatler et al. 2018), examples with small-bodied terrestrial taxa remain scarce (Brown et al. 2013; Hammond et al. 2016).

Among all of these “frontier-challenges,” the computational burden imposed by high-resolution ACT data remains as one of the most significant hurdles, particularly in validation studies on novel focal taxa (Resheff et al. 2014; Chimienti et al. 2016). Advanced machine learning techniques are required for interpretation of ACT signals, and their implementation commonly requires the expertise of an experienced data scientist (Ladds et al. 2017). An encouraging recent development is that a diversity of relatively simple algorithms has been found to perform similarly to, and, in some cases, outperform, more complex approaches (associated with unsupervised modeling) (Collins et al. 2015; Tatler et al. 2018; Patterson et al. 2019). Additionally, time-matched observations of behavior for supervised model training procedures were shown to significantly improve model performance (Bom et al. 2014; Sur et al. 2017). Nevertheless, a set of best practices or standard procedures for managing, processing, and analyzing these data has yet to emerge, and the idiosyncratic nature of individual study systems plays a prominent precluding role (Chimienti et al. 2016). While numerous details will undoubtedly remain system-specific, ACT studies must provide detailed, step-wise procedures, especially in case studies with novel focal organisms.

A strength of ACTs and other bio-logging technologies is the ability to use data from multiple simultaneously logging or transmitting devices to collectively inform animal behavior and ecology. Such integrative frameworks are increasingly being used by behavioral ecologists and eco-physiologists to function as a “daily diary” for individual animals (Wilson et al. 2008; Walker et al. 2015; Wilmers et al. 2015). The urgency to broaden the diversity of organisms and study systems suitable for these frameworks is now enhanced when considering the potential effects of ongoing global change phenomena, and applications with species of significant conservation concern have illustrated the important ability of these techniques to inform management strategies (Wilson et al. 2008; Wilson et al. 2017; Kienle et al. 2019; van Beest et al. 2019). Accordingly, these technologies and their associated processing and analysis protocols will become increasingly important tools for scientists in the race to improve our understanding of organismal responses to environmental conditions.

The overarching goal of this dissertation is to develop and validate an integrative framework combining radio telemetry and accelerometry protocols for long-term monitoring of movement behavior in small-bodied and secretive terrestrial vertebrates. To achieve this, I selected a model organism (Western Diamond-backed Rattlesnakes, *Crotalus atrox*) for a group (snakes) that has been studied extensively with radio telemetry but is completely overlooked in applications with advanced bio-logging technologies, such as accelerometers (ACTs). Despite their general absence from bio-logging studies, snakes, and pitvipers (Viperidae: Crotalinae) in particular, represent intriguing model organisms, particularly when considering interactions between behavior and environmental conditions. As ectotherms, snake behavior is tightly linked to external factors (Beaupre 2009). Because of extreme low-energy specialization, many snakes are also often numerically dominant relative to avian and mammalian predators in their

respective systems (Willson and Winne 2015), facilitating the manageable study of a large sample within a relatively small area. Lastly, pitvipers, specifically, are highly amenable to internal implantation procedures (Reinert and Cundall 1982), allowing long-term field monitoring via radio telemetry and concurrent data-logging devices. Significant gaps in our knowledge of behavior and ecology remain for most snake species because of their highly secretive life histories (Dorcas and Willson 2009), and although efforts have been made to improve our ability to monitor snake movement and behavior in the field, such as the development of automated very high frequency (VHF) telemetry arrays (Ward et al. 2013; Sperry et al. 2013; DeGregorio et al. 2015), these approaches are constrained by snake proximity to fixed-point antennas. This proximity constraint is also exacerbated in study sites with considerable physical barriers to NHF signals, such as those in mountainous terrain. The methodological framework developed herein circumvents many of the limitations associated with using radio telemetry to measure activity through remote and continuous recording unlimited by observer presence, time of day, or scale of space use. However, ACTs do not replace radio telemetry as the most useful technique for longitudinal monitoring of spatial ecology in snakes. Therefore, this dissertation aims to illustrate how these two technologies can be integrated to simultaneously provide data on the temporal and spatial dimensions of snake movement behavior for improved testing of proximate and ultimate drivers of snake behavioral decisions. Furthermore, the robust data collection, processing, and analysis methods described herein are intended to be highly flexible and easily transferred to other small, secretive species that aren't amenable to most other bio-logging technologies.

## 1.1 Primary Objectives

- 1) Validate data collection and processing procedures for long-term, low-frequency ACT field-monitoring with *C. atrox* as a model for other small-bodied, secretive terrestrial vertebrates in remote or inaccessible habitats
  - a. Integrate radio telemetry and tri-axial accelerometry protocols
  - b. Train and test supervised machine learning models to achieve accurate automated classification of coarse behavioral mode (i.e. “active” vs. “inactive”)
  - c. Demonstrate potential utility of RT-ACT framework in applied contexts by constructing continuous activity budgets at multiple temporal scales to evaluate patterns in the timing and duration of activity
- 2) Use radio telemetry to evaluate the interacting effects of sexual selection, behavioral season, and plasticity in movement and space use strategies by *C. atrox*
- 3) Further demonstrate the usefulness of pitvipers as model organisms for research in ecology and evolutionary biology

## **Chapter 2:** When, how, and why do secretive animals move? A framework integrating radio telemetry and accelerometry protocols for long-term activity monitoring with a cryptic pitviper

### **2.1 Abstract**

For many small, cryptic, and secretive animals, an understanding of the causes and consequences of movement decisions is often hampered by methodological limitations. The use of miniature accelerometer dataloggers for remote and continuous recording of animal activity in natural settings is becoming increasingly common. However, accelerometer applications are largely biased toward large-bodied species as a result of size limitations with smaller animals. We aimed to take advantage of ongoing miniaturization and advancement of bio-logging devices and associated computational techniques to develop a framework for long-term, low-frequency accelerometer monitoring of activity in small and secretive terrestrial species. We achieved this by internally implanting coupled radio transmitters and tri-axial accelerometers in rattlesnakes (*Crotalus atrox*) and conducting periodic field-validation observations of behavior. These observations were used to train and test supervised learning models (Random Forest, RF; Generalized Linear Elastic Net, GLMNET) for behavioral classification. The best performing RF model distinguished between periods of full-body “activity” and “inactivity” in rattlesnakes with high accuracy (activity = 96%, inactivity = 99%), and was applied to extensive full ACT datasets ( $94 \pm 99$  days,  $N = 12$ ) for automatized activity classification to produce activity budgets at multiple temporal scales. In general, these cryptic ambush predators were found to be highly

sedentary, with activity budgets being characterized by extensive periods of inactivity interrupted by punctuated bouts of activity. However, substantial inter-individual variation was also observed, as the proportion of time classified as “active” per individual dataset ranged from 1.6% to 37.1%. The same general daily activity pattern was conserved across all active seasons (spring, summer non-mating, summer mating), fall) with the majority of activity occurring during the evening or nocturnal diel periods. Contrary to activity timing, activity duration within diel periods displayed seasonal variation with activity increasing during the summer-mating season, possibly in mate-searching efforts. Concurrent recording of high-resolution activity data for a sample of individuals in nature was previously unattainable for most snakes and other small, secretive species. The RT-ACT framework combines simplicity and objectivity to provide a flexible set of widely-applicable data collection and processing procedures. Moving forward, improved temporal overlap among monitored individuals should enable applied extensions of this framework to evaluate potential internal and external drivers of fine-scale activity decisions. Additionally, independent captive behavioral observations can also be conducted to enable classification of additional cryptic behaviors that are rarely observed in wild-ranging individuals. Refining the presented classification scheme would provide a foundation for future investigations using the RT-ACT framework to establish relationships between individual behavioral decisions and performance, a key step towards placing secretive study species in theoretical contexts or linking behavioral ecology and eco-physiology to population dynamics.

## 2.2 Introduction

When, how, and why an animal moves are fundamental aspects of its biology and ecology, as activity patterns reflect the complex interplay between intrinsic (e.g., sex, physiological state, motivational state) and extrinsic (e.g., environmental conditions, predation, competition) factors that shape behavioral decisions (Shepard 2008; Nathan et al. 2008). Movement also has significant implications for biodiversity conservation, representing a critical mechanism in dispersal, inbreeding avoidance and gene flow, pathogen transmission, and responses to habitat modifications and other global change phenomena (Holyoak et al. 2008; Nathan et al. 2008; Jeltsch et al. 2013). Many modes of animal movement exist; such as migration, mate-searching, defending territories, and foraging (Clobert et al. 2012), and a key characteristic of these different motivational states is the variety of scales (spatial and temporal) at which they can occur. Migrations might be distinctly seasonal, occur over days or months, and span from hundreds of meters to over 1,000 kilometers depending on the taxon (Jeltsch et al. 2013), whereas foraging movements often occur in multiple bouts over a single day and usually within an animal's home range or territory (Jeltsch et al. 2013). Consequently, to properly characterize the diversity of movement behaviors for a species or population as well as understand the roles of various internal and external factors in specific strategies, it is essential to monitor activity across multiple scales (Trierweiler et al. 2012; Bauder et al. 2016).

Until very recently, studying animal movement behavior had been methodologically challenging for many taxa, as barriers associated with small body sizes, secretive life histories, adverse environmental conditions, and observer presence all combined to constrain direct observation or the application of many advanced monitoring technologies (Brown et al. 2013).

Since its original application with wildlife in the 1960s, direct observation via radio telemetry was the most commonly employed method for measuring the movement behavior of wild-ranging animals (Martin et al. 1993; Kays et al. 2015), restricting behavioral observation to a small fraction of daily activity. A combination of recent conceptual and technological advances in animal-borne data-logging devices (i.e., “bio-loggers”) has greatly improved the ability to identify causes and consequences of individual movement strategies, driving the emergence and rapid growth of the field of movement ecology (Nathan et al. 2008; Kays et al. 2015). Modern animal bio-logging technologies have circumvented many of the traditional limitations in movement ecology by enabling remote collection of large quantities of data on individual biology, physiology, and behavior (Brown et al. 2013; Kays et al. 2015). These devices have effectively given researchers the ability to remotely measure aspects of animal biology and ecology in the field at scales and precision that was historically restricted to laboratories (Nathan et al. 2008; Wilmers et al. 2015).

Miniaturized accelerometers are one of the most widely used animal-borne datalogger technologies (Shepard et al. 2010; Brown et al. 2013). Accelerometers (ACTs) allow remote and continuous monitoring of animal activity free from limitations of observer presence, terrain, weather, time of day, and scale of space use (Yoda et al. 1999; Wilson et al. 2006; Shepard et al. 2008; Brown et al. 2013). To date, most applications with ACTs and wild-ranging animals have involved large-bodied taxa and for those which direct observation is severely limited or impossible (Brown et al. 2013; Hammond et al. 2016), such as freshwater and marine fish (Murchie et al. 2011), marine mammals (Sato et al. 2003), and pelagic birds (Brown et al. 2013). However, both large (Grünewälder et al. 2012; Williams et al. 2014) and small terrestrial mammals (Hammond et al. 2016), tortoises (Lagarde et al. 2008), and large toads (Halsey and



White 2010) have all been subjected to ACT monitoring, and ongoing miniaturization of ACT devices and associated batteries continues to diminish weight restrictions for smaller species. Advancement in sensor technology has now set the stage for researchers to expand the diversity of organisms used in ACT applications. However, significant computational challenges and a general lack of standardization in processing and modeling techniques remain for ACT data (Collins et al. 2015), and this paucity of standard procedures further complicates pilot studies with new study organisms. Although the idiosyncratic nature of individual study systems and species can reduce the transferrable potential of specific protocols, a general methodological framework can still contribute to standardization of broader procedural checkpoints, such as field or lab-based observation approaches, model training procedures, and specific classification modeling techniques. It is therefore essential that ACT studies provide detailed, step-wise descriptions of the full data collection, processing, and modeling process.

Snakes are one vertebrate group that has been completely overlooked in animal-borne bio-logger applications. As highly secretive and often-nocturnal animals, significant gaps exist in our knowledge of snake biology, ecology, and behavior (Dorcas and Willson 2009). Several challenges unique to snakes account for their absence from advanced bio-logging studies, the most prominent being periodic ecdysis prohibiting long-term external attachment of loggers and morphological limitations (i.e., small body sizes of most species, and linear body plan) on the implantation of often-bulky data-logging devices. The original use of radio transmitters for longitudinal monitoring of snakes in the 1980s was transformative to field studies of snake ecology (Reinert and Cundall 1982; Reinert 1992; Beaupre 2016). However, coarse measures of activity based on Euclidean distances between relocation points hinder examination of mechanisms that shape activity patterns at different temporal scales (Whitaker and Shine 2003;

Lindstrom et al. 2015). Automated receiving units (ARUs) have been used to obtain continuous movement data for snakes (Davis et al. 2008; Sperry et al. 2013), but spatial limitations exist with this method (i.e., snake proximity to ARU), and study sites with high topographic relief (e.g., physical barriers to VHF radio signals) exacerbate this constraint. Herein, we aimed to validate long-term, low-frequency (1-Hz) ACT monitoring of activity in the field with Western Diamond-backed Rattlesnakes (*Crotalus atrox*). This data collection, processing, and analysis pipeline is intended to provide a general framework for long-term activity monitoring for small, secretive terrestrial animals inhabiting remote or largely inaccessible terrain. We make use of extensive field validation observations of behavior to train supervised learning models for automated classification of coarse behavioral mode (active movement vs. inactive). Using optimized models, we establish and evaluate fine-scale activity budgets for wild-ranging *C. atrox*.

## **2.3 Methods**

### **2.3.1 Field Data Collection**

We developed a streamlined data collection, processing, and analysis pipeline for long-term ACT monitoring in small, cryptic, and secretive terrestrial animals (Fig. 2.1). Radio transmitters (Holohil Systems Ltd., Model SB-2T) and tri-axial ACTs (Technosmart Europe srl., AXY-3, AXY-Depth) were coupled and internally implanted in study individuals (Reinert and Cundall 1982) and securely sutured to a rib (Hardy and Green 1999, 2000) in the same position and orientation for all individuals. Implants (SB-2T = 5 g; AXY = 2.5–7 g) comprised < 3% of

each individual's body mass at the time of implantation. Radio transmitters had a battery life of 10 months, and ACTs had data storage capacity (1Gb) that allowed logging for approximately 9 months when recording at 1-Hz. Since our goal was to conduct long-term recording of coarse behavioral mode ("active" vs. "inactive") in the field, we selected a low monitoring frequency for our ACTs (1-Hz) to minimize the trade-off between recording frequency and logging duration. Recent work has demonstrated that high classification accuracy can be achieved with low-frequency monitoring, especially when paired with extensive validation data (i.e., time-matched observations of behavior) (Tatler et al. 2018). Data were stored in the device's microprocessor until downloaded via USB connection following extraction from rattlesnakes.

All data collection procedures took place on Indio Mountains Research Station (IMRS) in Hudspeth County, Texas from June 2016–August 2018. More detailed accounts of the study site can be found in Worthington et al. (2019) and Mata-Silva et al. (2018). Because all study snakes were included in concurrent monitoring of spatial ecology via hand-held radio telemetry, a variety of spatial metrics were calculated to evaluate possible relationships between spatial data and activity data. These metrics included movement rate (meters per day, MPD), calculated as the straight-line distance between successive relocation points for an individual, distance per movement (DPM), calculated as the mean straight-line distance between relocation points that were  $\geq 5$  m apart, and minimum movement frequency (MMF), calculated as the number of movements ( $\geq 5$  m) made by an individual out of  $N$  relocations in a specifically defined time period. Rattlesnakes were relocated at least twice weekly via radio telemetry, at which time individuals were observed for visual validation of ACT signals. These field-validation observations were typically 30 min. in length and occurred at various times of the day (i.e., morning, mid-day, evening, and night) to encompass both active and inactive periods for

rattlesnakes. Hand-held video of surface behavior was recorded for later detailed inspection and manual behavioral annotation of ACT training data. All field observations were conducted independently by DLD.

### 2.3.2 Data Processing and Summary Statistics Selection

We selected two broad behavioral categories for classification (“activity” and “inactivity”). During field observations of ACT-equipped individuals, “activity” was classified as outstretched, full body movement. “Inactivity” was an observation of a snake (independent of body posture) that was not in full-body motion. To summarize acceleration data and make relationships between ACT signals and behavioral mode more discernable, we calculated a suite of 23 descriptive statistics across the  $x$  (forward and backward motion),  $y$  (side-to-side motion), and  $z$  (upward and downward motion) ACT axes. These summary stats were initially calculated to be “rolling” over varying temporal window sizes (one-second, 10-second, 20-second, and 1-min. period basis) to identify the optimal scale at which to classify activity with the machine learning models. We found a temporal window of 20 sec. to be the “goldilocks” scale in our classification scheme. Rattlesnake movement behavior is often characterized by frequent and relatively brief pauses attributed to chemo-sensory probing, and, therefore, very fine (1-sec.) and very coarse (1-min.) windows could potentially under and over-estimated periods of active movement, respectively. Additional ACT studies have presented similar results (Ladds et al. 2017), whereas others have opted for longer windows up to 10 min. in length to improve overall accuracy of behavior classification (Diosdada et al. 2015). Appropriate window size is highly context-dependent, as study species, targeted behaviors for classification, ACT recording

frequency, and the selected algorithms all combine to influence the optimal scale for classification (Jeantet et al. 2018). The “moving” or “rolling” statistics calculated here include the mean (average), standard deviation, minimum, maximum, and slope of a regression model. We also calculated Overall Dynamic Body Acceleration (ODBA) and Vectorial Dynamic Body Acceleration (VeDBA) to summarize signals using measures from the  $x$ ,  $y$ , and  $z$  axes. The ODBA and VeDBA measures have been widely used for summarizing acceleration in previous studies (Wilson et al. 2006; Shepard et al. 2008; Bidder et al. 2012), but their usefulness with the unique morphology and movement mechanics of snakes is untested. ODBA is the absolute sum of the dynamic body acceleration (DBA) along the  $x$ ,  $y$ , and  $z$  axes.

$$\text{ODBA} = |\text{DBA} \cdot x| + |\text{DBA} \cdot y| + |\text{DBA} \cdot z|$$

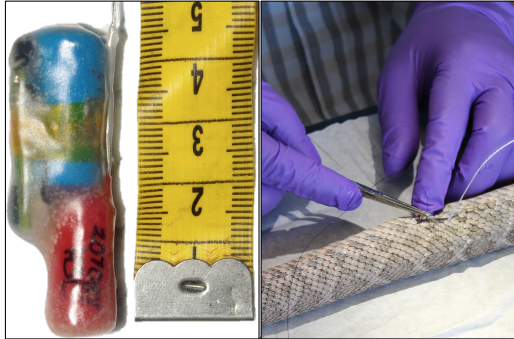
While VeDBA is the vector of DBA along the  $x$ ,  $y$ , and  $z$  axes.

$$\sqrt{\text{DBA} \cdot x^2 + \text{DBA} \cdot y^2 + \text{DBA} \cdot z^2}$$

Dynamic body acceleration is the raw acceleration value remaining after the removal of static acceleration due to gravity. Static acceleration was a constant subtracted (-1) from the axis oriented in the “upward and downward” plain, which was the  $z$  axis, in the case of our ACT implantation orientation (C. Catoni [Technosmart Europe srl.], personal communication; Brown et al. 2013). The relative importance of different summary statistics in the classification process was visualized by a variable importance plot for the best performing model. Variable importance was measured via mean decreases in the Gini Coefficient (Breiman 2002; Cutler et al. 2007).

Mean decrease in Gini measures how much each variable (summary stat) contributes to the homogeneity of the classification decisions by the model. The coefficient ranges from zero (homogeneous) to one (heterogeneous) and changes in Gini are summed for each variable and averaged at the end of the classification process. Variables that result in classifications with higher precision have a higher decrease in Gini coefficient.

### Field Data Collection



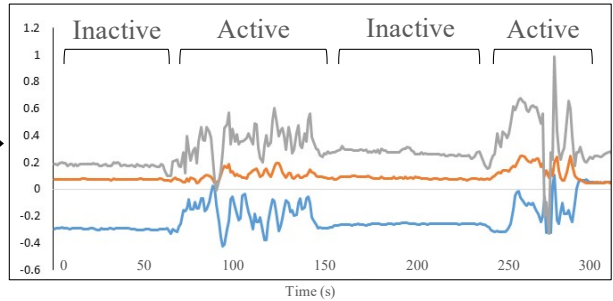
**Field Validation Observations** via radio telemetry relocation



**Device Extraction and Raw Acceleration Data Retrieval**

**Manual behavioral annotation** of field observation periods to create training dataset

### Supervised Model Validation



#### **Feature Extraction**

- Identify Optimal Temporal Window for classification of targeted behaviors
- Calculate “Rolling” Summary Statistics for ACT data

#### **Model Training**

Random Forest and GLMNET algorithms applied to annotated training dataset (hold-out data)

#### **Model Testing**

- Evaluate predictions made with hold-out data; calculate model performance; assess summary stat importance
- Repeat Feature Extraction and Model Training procedures to improve performance if necessary

### Model Application

**When, how, and why do secretive animals move?**  
Apply optimized model to full datasets to construct daily activity budgets

**Figure 2.1** Stepwise (*Field Data Collection, Supervised Model Validation, Model Application*) RT-ACT methodological framework for long-term, low-frequency accelerometer (ACT) data collection, processing, and analysis procedures validated herein. Details regarding each step in the framework can be found within methods sections 2.3.1–2.3.3.

### 2.3.3 Supervised Learning Models for Activity Classification

Because of the high-resolution logging of ACTs, the resulting datasets from field deployments are typically very large (herein, routinely exceeding one million data points per individual). As a result, manually labelling periods of behavior within full datasets is practically impossible, making automated labeling via machine learning techniques a critical step in ACT applications. The process of selecting the appropriate model for specific classification tasks can often be complicated and time consuming (Ladds et al. 2017), as a wide variety of algorithms are available for use in classification of behavior from ACT signals. These models range from relatively simple approaches like simple linear discriminant analyses (Nathan et al. 2012) and decision trees (Nishizawa et al. 2013), to more advanced techniques such as random forests (Lush et al. 2016; Ladds et al. 2017; Tatler et al. 2018), support vector machines (Hokkanen et al. 2011), gradient booster machines (Ladds et al. 2016; Ladds et al. 2017), artificial neural networks (Banerjee et al. 2012), and Gaussian mixture models (Chimienti et al. 2016). Here, we initially opted to use the  $k$ -nearest neighbor clustering algorithm, the random forest (RF), and generalized linear elastic net (GLMNET) to provide supervised models for activity classification of rattlesnake ACT signals. Supervised models make use of algorithms to “learn” (via model training) the relationship between a set of input variables (i.e., summary statistics) and output



variables (i.e., assigned classifications). After initial exploratory analyses, we quickly ruled out the  $k$ -nearest neighbor clustering approach, as significant variation within our two broad behavioral categories reduced its usefulness. We selected the RF and GLMNET algorithms as they are both reasonable approaches, but they assume different solutions to this classification problem. Random forest models have been used extensively in the ACT literature (Lush et al. 2016; Ladds et al. 2017), and often perform well in behavioral classification with a diversity of focal taxa (Tatler et al. 2018). Random forests make use of multiple learning algorithms simultaneously through bootstrapping to find a unified classification tree that corrects for overfitting and noise (Efron and Hastie 2016). We make use of the default split of  $\sqrt{p}$  for each bootstrapped tree as it decreases bias, but also results in relatively independent trees. This is a powerful methodology since it is relatively efficient and, because of the random selection of features of the data, should be more accurate (and less biased) than other learning algorithms. We also make use of a generalized linear model (GLM) with an elastic net penalty applied, provided we have a binary predictor and data that are highly correlated. In the GLM elastic net model (GLMNET), the regularization path has an elastic net penalty applied on a grid of values for the regularization parameter, lambda. This is intuitively appealing and efficient because it maximizes the estimating equation with respect to the regression parameters over a grid of reasonable values for  $\lambda$ .

$$\min_{\beta_0, \beta} \frac{1}{N} \sum_{i=1}^N w_i l(y_i, \beta_0 + \beta^T X_i) + \lambda \left[ (1 - \alpha) \frac{\|\beta\|_2^2}{2} + \alpha \|\beta\|_1 \right]$$

The tuning parameter  $\lambda$  controls the strength of the penalty and  $\alpha$  controls the type of penalty applied (ranging from a lasso penalty to ridge penalty). In the model training stage, we specified

the cross-validation folds and validated  $\lambda$  across a grid of values for  $\alpha$ . We called the elastic net function multiple times and averaged over the results while using the same fold vector for each call. This approach reduces noise and produces an optimum value for  $\alpha$ . After the optimal penalty was chosen using inner cross-validation loops, it was applied for modeling and prediction. This is preferred over simultaneously validating  $\alpha$  and  $\lambda$  because the tuning parameter is informatively selected in this approach and, hence, an optimal value for both parameters is more likely to be selected.

We used the RF and GLMNET models to provide a supervised learning model that we validated using holdout data (i.e., “test data”) prior to making predictions on the full training datasets. Once we illustrated model efficacy, we then used the full set of ACT data (field-collected datasets) for activity classification. The training data was substantial, with 42,224 observations at a 1-Hz resolution (1-Hz = one value recorded per-second along each axis). Using a 20% holdout sample, this allotted 33,713 observations for training and 8,511 for testing/validation. These data were derived entirely from the time-matched observations of behavior in the field. We assess the correspondence between observed and predicted behavioral mode for both models in a confusion matrix reporting classification accuracy, precision, and recall. We also report the training errors and test errors for the elastic net and random forest models. Accuracy is the overall proportion of positive classifications (i.e., predicted matches observed), precision is defined as the proportion of predictions that matched the observed behavior within each category (i.e., active and inactive), and recall is the proportion of observations within a behavior category that were correctly predicted as that behavior (Campbell et al. 2013). All random forest estimation was performed in *R* (R Core, 2017) using the

*randomForest* package (Liaw and Weiner, 2002), and all GLMNET estimations were also performed in *R* using the *glmnet* package (Friedman et al. 2010).

Following model validation procedures, we selected the best performing model to classify periods of activity and inactivity in the full dataset with 95,599,407 data points (1-Hz resolution). Using these behavioral predictions, we visualized activity budgets using radial histograms to explore daily activity patterns and evaluate intra- and inter-individual variation in activity timing, duration, and frequency. We also explored relationships between daily activity, season (winter [Dec–Mar], spring [Apr–May], summer non-mating [Jun–Jul], summer-mating [Aug–Sep], fall [Oct–Nov]), and diel period (morning [0601–1000 h], diurnal [1001–1800 h], evening [1801–2100 h], nocturnal [2101–0600 h]). After fitting a Generalized Linear Mixed (GLMM) effects model with these data, Least Squared (LS) mean comparisons with multiplicity adjustments were employed to test for potential differences in the timing and duration of activity within diel periods across seasons.

## **2.4 Results**

### **2.4.1 Field Deployment and Recovery**

In total, 19 rattlesnakes (17 male, 2 female) were implanted with RT-ACTs and released for field monitoring and recording between June 2016 and August 2018. Because of battery malfunctions in seven units (five ACT failures, two RT failures), only 12 datasets from all male *C. atrox* with sufficient data-recording durations were included in processing and analysis procedures. Recording durations for these 12 datasets ranged from 6 to 289 days (mean  $\pm$  SD = 94

$\pm 99$  days) for a cumulative total of 1,131 ACT recording days (Table 2.1). There were 152 independent field-validation observations conducted on these individuals, culminating in just under 32 hours of time-matched observations of behavior that were used in model training procedures. The number of observations vary considerably among individuals (Table 2.1), in part because of variable logging durations, but also the inability to confirm behavior during relocations when individuals were not visible. Manual annotation of ACT datasets was based on field video (when available) and detailed notes of validation observations. Time-matched behavioral labelling was conducted once independently by DLD (field observer) and a second time by a separate individual who was not present for the field observation (to minimize bias in interpretation of video or field notes). In rare cases where annotations were considerably different between labelers for a specific observation, those periods were re-evaluated and discarded if a clear behavioral classification could not be distinguished (often because of poor video quality or inconsistent visibility of the rattlesnake). Unsurprisingly, one of the primary motivations for developing ACT monitoring protocols for snakes (i.e., secretive life histories) proved to be a significant hurdle in this stage for validation, given that instances where snakes were observed actively moving on the surface were infrequent (1.2 of 32 hours), and even then, it was difficult to conduct extensive observations without disturbing the individual (often because of the use of flashlights during nocturnal observations). Fortunately, the long-term deployment of dataloggers yielded sufficient opportunities, but model training likely would have been less effective with short-duration deployments seen in many other ACT field studies of animal behavior that select higher monitoring frequencies.

**Table 2.1** Body size (Snout-Vent Length [SVL], Body Mass), ACT logging periods, and number of field validation observations for 12 male *C. atrox* included in processing and analyses. Note that fewer validation observations were conducted for rattlesnakes monitored during winter inactive periods, because of the frequent inability to visually confirm behavior. Body sizes included to illustrate the minimally invasive nature of coupled radio transmitter-accelerometer devices (12 g), as they comprised < 3% of each individual's body mass at the time of internal implantation.

Tag ID	SVL (mm)	Body Mass (g)	Logging Period (days)	Validation Observations
RT-ACT1	855	466	07/10/2016–08/01/2016 (22)	11
RT-ACT2	870	523	08/27/2016–02/23/2017 (180)	26
RT-ACT3	890	502	08/27/2016–09/02/2016 (6)	3
RT-ACT4	860	504	10/10/2016–02/15/2017 (128)	15
RT-ACT5	855	481	10/24/2016–05/30/2017 (218)	31
RT-ACT6	990	602	05/02/2017–06/15/2017 (44)	11
RT-ACT7	990	656	08/17/2017–9/12/2017 (26)	7
RT-ACT8	975	632	08/25/2017–09/09/2017 (15)	6
RT-ACT9	890	547	9/29/2017–06/25/2018 (289)	25
RT-ACT10	860	472	10/06/2017–10/22/2017 (16)	3
RT-ACT11	985	588	10/10/2017–04/03/2018 (175)	8
RT-ACT12	990	601	05/21/2018–06/02/2018 (12)	6
Mean ± s.d./Total	944 ± 91	613 ± 194	94 ± 99 days/1,131 days	152 observations

### 2.4.1 Validation of Supervised Machine Learning Models

The RF and GLMNET models were developed using the full set of training data. The GLMNET utilized a two-class logistic link function for the cross-validation and incorporates five folds and uses the optimum  $\lambda$  and  $\alpha$ . This is a strong approach considering the scale of the data and provided we are attempting binary classification. The RF models are arguably a less relevant approach than an elastic net GLM, but one used successfully for behavioral classification in other ACT applications (Lush et al. 2016; Ladds et al. 2017; Tatler et al. 2018). In the RF models, 1,000 random starts for bootstrap-based tree generations were utilized and proximity measures were saved for predictions. Both models (GLMNET and RF) were highly accurate at classifying known periods of activity and inactivity in the training data (Tables 2.2, 2.3), with the RF model slightly outperforming the GLMNET model. In general, models more accurately classified inactivity (RF = 99.3%, GLMNET = 97.7%) than activity (RF = 96.2%, GLMNET = 91.8%), which is not surprising considering the tremendous variation in raw ACT signals during activity (see sample ACT waveform data in Fig. 2.1). A variable importance plot was produced to illustrate the relative rank of each summary statistic used in the classification process for the optimized RF model (Fig. 2.2). Measures of data dispersion (standard deviation, maximum value) were the top five ranking variables, with the standard deviation of values on the  $x$ ,  $y$ , and  $z$  ACT axes being the most informative for distinguishing between periods of activity and inactivity identified in the training dataset.

**Table 2.2** Confusion matrix for the Random Forest and Generalized Linear Mixed-NET (GLMNET) models. Recall, precision, and accuracy reflects classification performance with full training dataset.

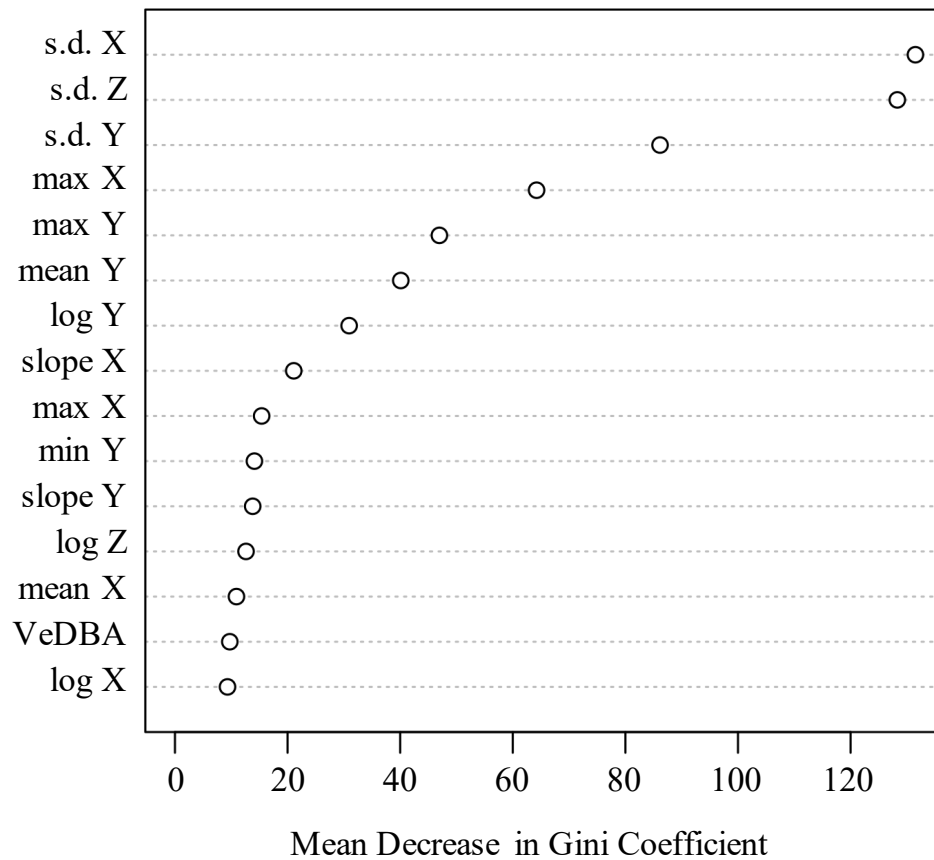
		<i>Predicted Behavior</i>			
<i>Observed Behavior</i>	Random Forest	Active	Inactive	Total	Recall
	Active	<b>4,211</b>	168	4,379	96.2%
	Inactive	240	<b>37,605</b>	37,845	99.3%
	Total	4,451	37,773	42,224	
	Precision	94.6%	99.6%		<b>Accuracy = 99.0%</b>
	GLMNET	Active	Inactive	Total	Recall
	Active	<b>4,020</b>	359	4,379	91.8%
	Inactive	870	<b>36,975</b>	37,845	97.7%
	Total	4,890	37,334	42,224	
	Precision	82.2%	99.0%		<b>Accuracy = 97.0%</b>

**Table 2.3** General Linear Mixed-elastic net (GLMNET) and Random Forest (RF) model specifications with the optimum  $\lambda$  and  $\alpha$  values as well as the number of folds for validation.

When reporting errors, the subscripts IA and A stand for “In-Active” and “Active.” The training error is the error using only the training data and the test error is the observed error using the validation set and observed movements. The sample sizes used for training and validation (denoted  $n_t$  and  $n_v$ ) are provided.

Training and Validation sample sizes	$\hat{\lambda}, \hat{\alpha}, n_{folds}$	GLMNET Test error	GLMNET Accuracy	RF Test error	RF Accuracy
$n_t = 33,713$	0.03, 0.00,	$\epsilon_{IA} = 0.038$	97.0%	$\epsilon_{IA} = 0.011$	99.0%
$n_v = 8,511$	10	$\epsilon_A = 0.025$		$\epsilon_A = 0.005$	

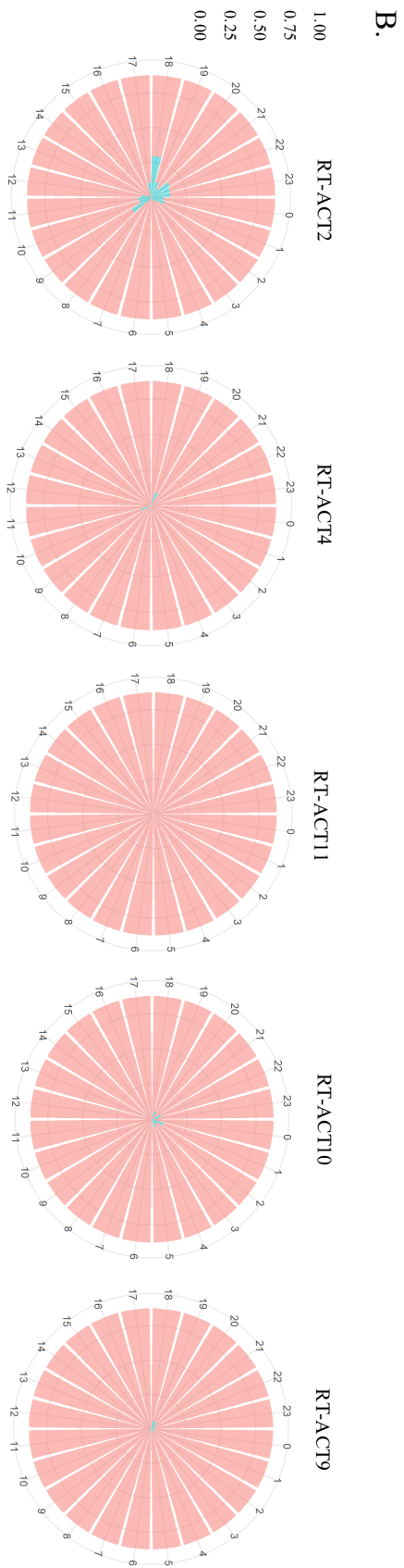
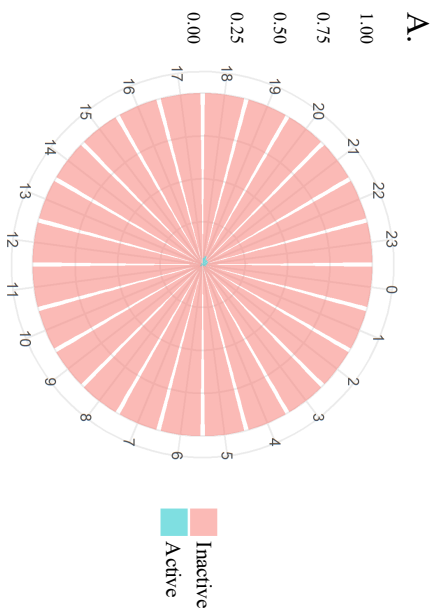




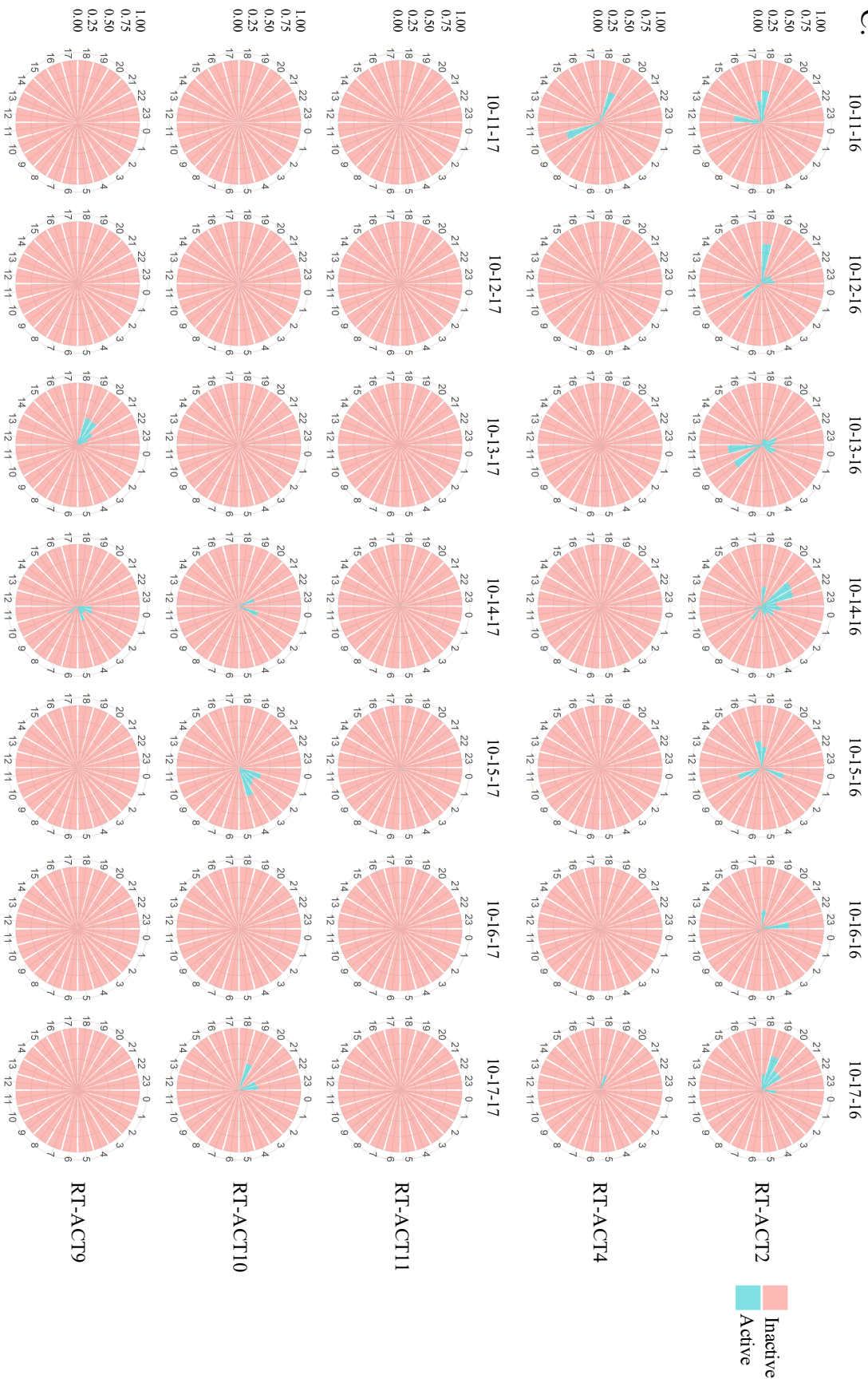
**Figure 2.2** Variable importance plot for optimized Random Forest model used for classification in full activity dataset. Y-axis depicts top 15 ranked variables (based on mean decrease in Gini, x-axis) out of 23 summary statistics calculated and used in model training. Note that measures of data dispersion (s.d., maximum) were the most informative variables for classification.

#### 2.4.2 Activity Budgets: Timing and Duration of Daily Activity

Overall, rattlesnakes were highly sedentary during logging periods (Fig. 2.3), although with considerable inter-individual variation, as overall proportions of time spent active per-individual (over entire logging period) ranged from 1.6% to 37% (mean  $\pm$  s.d.;  $0.135 \pm 0.139$ , Table 2.4). There was some noticeable variation in the proportion of time spent active within diel periods across seasons, particularly in regard to the increase in activity duration during the summer mating season (Table 2.4; Fig. 2.4). However, LS mean comparisons with multiplicity adjustments revealed no significant differences in activity timing and duration (relative to diel period) within and between active seasons (spring, summer non-mating (NM), summer mating (M), fall). The only significant differences detected in diel period activity were between the inactive (winter) and active seasons (Table 2.4; Fig. 2.4).



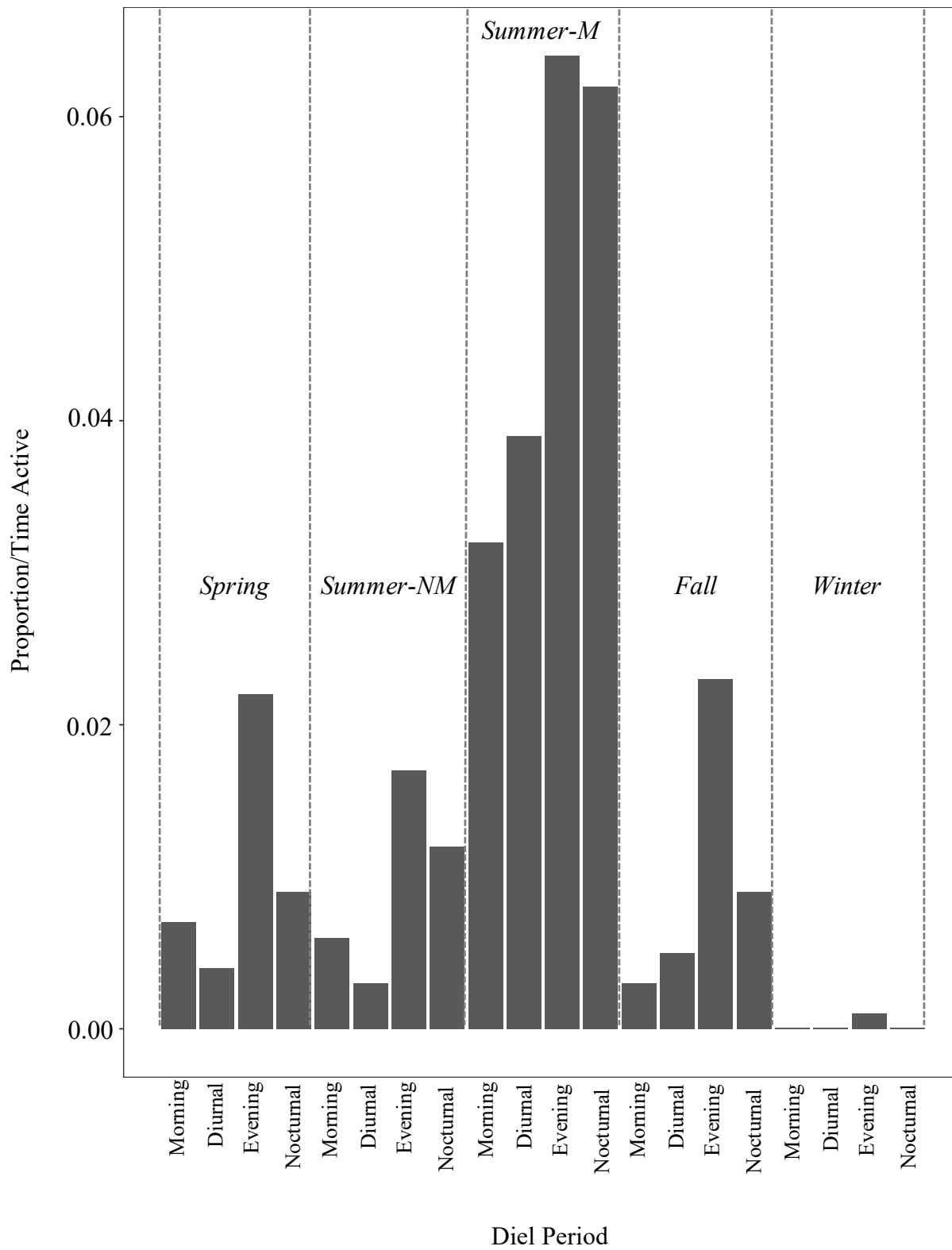
C.



**Figure 2.3** Radial histograms depicting activity budgets at monthly (A, B) and daily scales (C) for male *Crotalus atrox* monitored during October 2016 and 2017. Proportions are derived from classifications made by the optimized RF model. Individual bars correspond to a single hour within a 24 h period. The aggregated plot (A) depicts the mean activity budget for all rattlesnakes monitored during October ( $N = 6$ ). The individual monthly plots (B) depict the mean daily activity budgets for each of the five *C. atrox* that contributed data across every day of October ( $N = 5$ ). The daily plot mosaic (C) depicts the proportion of time spent active (blue) or inactive (pink) within each hour of individual days during the week of October 11–October 17 (2016 or 2017). October was selected for visualization because it had the largest concurrent ACT monitoring sample size among the active season months (Apr–Oct) during the study. Note that although periods of activity are highly infrequent and often temporally dispersed between extensive periods of inactivity, there is considerable snake-to-snake variation in patterns.

**Table 2.4** Mean proportions of time spent active within diel periods and seasons over entire ACT logging period for *C. atrox* ( $N = 12$ ). Seasons consisted of spring [Apr–May], summer non-mating [Jun–Jul], summer-mating [Aug–Sep], fall [Oct–Nov]), and winter [Dec–Mar]. Diel periods were: Morning [0601–1000 h, Diurnal [1001–1800 h], Evening [1801–2100 h], Nocturnal [2101–0600 h]). Least Squared (LS) means used in multiple comparisons and 95% Confidence Intervals are also provided. The only significant between-season differences in diel period activity proportions (logit transformed) involved the Summer-Mating and Winter seasons (Morning:  $P = 0.002$ , Diurnal:  $P = 0.03$ , Evening:  $P = 0.008$ , Nocturnal:  $P = 0.01$ ), and Summer-Non-Mating and Winter (Nocturnal:  $P = 0.04$ ). The highest mean activity durations were found within the evening diel period across all active seasons, followed by the nocturnal diel period.

<b>Diel Period/Season</b>	<b>Mean Activity Proportion <math>\pm</math> s.d.</b>	<b>LS Mean <math>\pm</math> s.e.</b>	<b>95% Confidence Interval (<i>lower–upper</i>)</b>
Morning/Spring	$0.007 \pm 5.541\text{e-}5$	$-5.18 \pm 0.89$	$-6.92 - -3.44$
Morning/Summer-NM	$0.006 \pm 2.111\text{e-}4$	$-5.63 \pm 0.89$	$-7.36 - -3.89$
Morning/Summer-M	$0.032 \pm 1.006\text{e-}4$	$-4.46 \pm 0.72$	$-5.88 - -3.05$
Morning/Fall	$0.003 \pm 2.565\text{e-}5$	$-6.45 \pm 0.89$	$-8.19 - -4.72$
Morning/Winter	$< 0.001 \pm 5.127\text{e-}6$	$-8.60 \pm 0.63$	$-9.83 - -7.38$
Diurnal/Spring	$0.004 \pm 2.828\text{e-}5$	$-5.64 \pm 0.89$	$-7.38 - -3.90$
Diurnal/Summer-NM	$0.003 \pm 4.596\text{e-}5$	$-6.44 \pm 0.89$	$-8.18 - -4.71$
Diurnal/Summer-M	$0.039 \pm 1.631\text{e-}4$	$-4.70 \pm 0.72$	$-6.12 - -3.28$
Diurnal/Fall	$0.005 \pm 2.429\text{e-}5$	$-5.59 \pm 0.89$	$-7.33 - -3.86$
Diurnal/Winter	$< 0.001 \pm 6.653\text{e-}6$	$-7.57 \pm 0.63$	$-8.80 - -6.34$
Evening/Spring	$0.022 \pm 1.113\text{e-}4$	$-3.75 \pm 0.89$	$-5.49 - -2.02$
Evening/Summer-NM	$0.017 \pm 3.338\text{e-}4$	$-3.95 \pm 0.89$	$-5.69 - -2.22$
Evening/Summer-M	$0.064 \pm 1.986\text{e-}4$	$-3.84 \pm 0.72$	$-5.26 - -2.42$
Evening/Fall	$0.023 \pm 8.690\text{e-}5$	$-4.02 \pm 0.89$	$-5.76 - -2.28$
Evening/Winter	$0.001 \pm 1.542\text{e-}5$	$-6.87 \pm 0.63$	$-8.10 - -5.64$
Nocturnal/Spring	$0.009 \pm 4.271\text{e-}5$	$-4.60 \pm 0.89$	$-6.34 - -2.87$
Nocturnal/Summer-NM	$0.012 \pm 1.911\text{e-}4$	$-4.37 \pm 0.89$	$-6.11 - -2.63$
Nocturnal/Summer-M	$0.062 \pm 9.560\text{e-}5$	$-3.87 \pm 0.72$	$-5.28 - -2.45$
Nocturnal/Fall	$0.009 \pm 3.260\text{e-}5$	$-5.20 \pm 0.89$	$-6.93 - -3.46$
Nocturnal/Winter	$< 0.001 \pm 4.929\text{e-}6$	$-8.23 \pm 0.63$	$-9.46 - -7.00$



**Figure 2.4** Activity durations (bars denote mean proportion of time classified as “active” by RF model) within diel periods and seasons over entire ACT logging period for *C. atrox* ( $N = 12$ ). Seasons consisted of: Spring [Apr–May], Summer Non-Mating (NM) [Jun–Jul], Summer-Mating (M) [Aug–Sep], Fall [Oct–Nov]), and Winter [Dec–Mar]. Diel periods were: Morning [0601–1000], Diurnal [1001–1800], Evening [1801–2100], Nocturnal [2101–0600]). During the active seasons (spring, summer, fall), activity patterns follow the same general pattern with higher proportions of time spent active during the evening and nocturnal periods relative to the morning and diurnal periods. Although there is a noticeable increase in activity duration during the summer-mating season (summer-M), those proportions were not significantly different than those during other active season diel periods (spring, summer non-mating (summer NM), fall).

## 2.5 Discussion

By internally implanting coupled radio transmitters and tri-axial accelerometers in rattlesnakes, we achieved two important goals during this project: 1) we conducted validation observations of behavior in the field, rather than in potentially restrictive captive settings (Shuert et al. 2018), to provide time-matched behavioral data for training and testing supervised machine learning models; and 2) we automatically labeled coarse behavioral mode (active vs. inactive) in extensive field datasets ( $94 \pm 99$  days) in order to construct long-term activity budgets (Fig. 2.3) and evaluate patterns in the timing and duration of activity at multiple temporal scales. The high classification accuracy achieved for both the GLMNET (97.0%) and RF models (99.0%) validates our general methodological framework (Fig. 2.1) and illustrates how combining radio telemetry and accelerometry protocols with advanced machine-learning techniques can enable



high model performance in behavior segmentation for small, secretive, terrestrial study species at very low monitoring frequencies (1-Hz). Critical, in this regard, were the periodic field-validation observations of behavior, which, in addition to providing the time-matched behavioral data for model training and testing, also made our models robust to inter-individual variability in ACT signals (Nathan et al. 2012; Bom et al. 2014; Hammond et al. 2016; Sur et al. 2017). This latter detail was particularly important for this study, given that RT-ACTs were internally implanted, which prevented visual confirmation of consistent logger positioning during field deployments. During extraction procedures, we did not document any instances of significant logger repositioning within the body cavities of rattlesnakes, indicating that suturing RT-ACTs to a rib had successfully affixed implants over the entire recording duration. Nevertheless, field validation observations are an important additional safeguard against subtle variation in ACT orientation, which can have significant effects on ACT signals within and between individuals and is a potential source of error in relating signals to behavior (Brown et al. 2013; C. Catoni [Technosmart Europe srl.], personal communication).

Although both algorithms performed well within our classification scheme, the RF model slightly outperformed the GLMNET (Tables 2.2, 2.3). This was somewhat surprising, given that the GLMNET was selected as an ideal algorithm for this study because of its proficiency in binary classification. Both models were more accurate at classifying the “inactive” behavioral mode in rattlesnakes (Table 2.2), likely as a result of the relative scarcity of “active” observations in the training dataset (1.2 of 32 hours) and the substantially greater variation in ACT signals seen during periods of active movement. While RFs remain computationally intensive to train (Cutler et al. 2007), they have now been used to successfully classify behavior from ACT signals in a wide variety of terrestrial and aquatic taxa (reviewed in Shuert et al.

2018). However, the vast majority of such studies, similar to ours, found it difficult to achieve high accuracy with a large number of behavioral modes and often opted for classifying fewer categories (i.e., two to four) (Shuert et al. 2018). For this project, we were restricted to classifying two coarse behaviors (activity and inactivity) largely because of limited observations of other cryptic behaviors during validation observations. Within this scheme, the most important variables (summary statistics) for classification were consistently identified as the standard deviation of the DBA along the  $x$ ,  $y$ , and  $z$  axes (Fig. 2.2). Further, the  $x$  and  $y$  axes were generally more informative than the  $z$  for activity classification, likely because of the general lack of upward and downward ( $z$ ) movement involved in rattlesnake locomotion (particularly in the posterior third of the body where ACTs were implanted). Some infrequently observed behaviors, such as male combat or predatory/foraging behaviors, might involve movements that would be best distinguished using signals on the  $z$  axis. Anterior placement of ACTs could also enable classification of several additional behaviors, but this would either involve external attachment, which is impractical because of periodic ecdysis and a high potential for behavioral obstruction, or subcutaneous implantation (requiring a very small, low-profile battery). The ODBA and VeDBA measures were not as informative as standard measures of data dispersion (s.d., range, etc.) for the distinction between activity and inactivity (Fig. 2.2). These two metrics are widely used in ACT studies as a proxy for energy expenditure in free-ranging animals (most often in terrestrial quadrupeds) (Wilmers et al. 2015) and have been shown to correlate well with the overall “intensity” of different movement behaviors (Tatler et al. 2018). Though their usefulness for studying snake energetics is limited by the fact that ectotherms exhibit energetically important thermoregulatory behaviors that don’t involve body movement, both

measures could prove to be more informative for classification given a scheme involving additional behavior modes that vary in intensity (Tatler et al. 2018).

Long-term activity budgets allowed for detailed visualization (Fig. 2.3) and examination of the timing and duration of activity. In general, *C. atrox* activity was found to be highly punctuated and infrequent, characterized by extensive bouts of inactivity between brief periods of activity (Fig. 2.3). Considerable inter-individual variation was also observed (Fig. 2.3), which is characteristic of most high-resolution bio-logging data. Overall (entire logging period) proportions of time spent active per-individual range from 1.6% to 37% (Table 2.4). As ectothermic, ambush foraging, low-energy specialists, pitvipers are known to spend extensive periods of time waiting motionless for potential prey to pass within striking distance (Clark 2016). Numerous radio telemetry studies on rattlesnakes (Reinert and Cundall 1982; Reinert 1992; Roth 2005; Blouin-Demers et al. 2007; Glaudas and Rodríguez-Robles 2011), including the spatial data collected from *C. atrox* during this study (see Chapter 3), corroborate these general findings, as pitvipers can often exhibit impressive displays of inactivity but can also make substantial long-range movements within large home ranges (in some cases we observed movements greater than 1 km in under 24 h). Evaluation of concurrent spatial data from RT-ACT *C. atrox* show positive relationships between movement rate (meters-per-day, MPD) and movement frequency (MMF) and the proportion of time spent active over entire ACT logging durations for individual rattlesnakes (MPD: adjusted  $R^2 = 0.56$ ,  $P < 0.01$ ; MMF: adjusted  $R^2 = 0.63$ ,  $P < 0.01$ ; Table 2.5). Conversely, there was no significant relationship between distance-per-movement and activity (adjusted  $R^2 = -0.05$ ,  $P = 0.49$ ). Although we are cautious of drawing conclusions from these results considering the limited sample size, the general correspondence between spatial metrics and ACT-derived activity further validates the classifications provided

by the RF model. Additionally, by simultaneously informing the temporal and spatial aspects of movement ecology, there is potential for these distinct data types to be complementary, much like animal-attached GPS dataloggers and ACTs are merged in other bio-logging studies (Wilson et al. 2008; Berlincourt et al. 2015; Walker et al. 2015). Combining spatial data with high-resolution activity data has provided important insights with larger study organisms, such as identifying different behavioral states along movement paths by foraging animals (Nams 2014). RT-ACTs could allow detection of temporal or spatial shifts in behavior by small, secretive species that would have been overlooked without an integrated approach (Nathan et al. 2012).

As with other desert-dwelling ectotherms, surface activity in *C. atrox* is largely dictated by ambient temperature. At our study site in the arid northern Chihuahuan desert, we expected daily and seasonal activity patterns to largely reflect this constraint, with the majority of activity occurring during nocturnal periods in the warmer summer months (Jun–Sep) and a more cathemeral (i.e., morning, evening) activity pattern during the comparatively cooler spring (Apr–May) and fall (Oct–Nov) months. We also expected greatly reduced activity by rattlesnakes during the winter inactive season (Dec–Mar). Counter to our predictions, rattlesnakes displayed the same general daily activity pattern across all active seasons (spring, summer (NM, M), fall) with the majority of activity occurring during the evening or nocturnal diel periods (Table 2.4). While this general pattern in the timing of activity did not vary seasonally, the mean proportion of time spent active within diel periods does vary somewhat, as activity duration increases during the summer-mating season (Fig. 2.4). Unsurprisingly, activity is greatly reduced during all diel periods in winter. The apparent increase in activity by male *C. atrox* during the summer-mating season corresponds with numerous observations of male pitvipers elevating movement during mating seasons in mate-searching efforts (Duval et al.

1993; Madsen et al. 1993; Duvall and Schuett 1997; Glaudas and Rodríguez-Robles 2011).

However, it is important to note that the margins between activity durations discussed here are remarkably thin (i.e., mean proportion of time spent active within diel periods ranges from < 0.01% to 6.4%). For instance, the highest recorded mean activity duration was within the evening diel period during the summer-mating season, where, on average, individuals were active for 11.52 out of 180 min. Therefore, we are cautious of drawing conclusions from these results beyond using them as further validation for our RT-ACT framework.

**Table 2.5** Concurrent spatial metrics and accelerometer (ACT)-derived activity proportions for entire ACT-logging period. Meters Per Day (MPD) (log base 10) and Minimum Movement Frequency (MMF) (log base 10) were positively related to activity (proportion of time classified as “active” over logging period) (MPD: adjusted  $R^2 = 0.56$ ,  $P < 0.01$ ; MMF: adjusted  $R^2 = 0.63$ ,  $P < 0.01$ ), while Distance Per Movement (DPM) (square root) was not related to activity (adjusted  $R^2 = -0.05$ ,  $P = 0.49$ ).

Tag ID	Logging Period (days)	MPD	DPM	MMF	Activity
RT-ACT1	07/10/2016–08/01/2016 (22)	5.37	25.5	0.22	0.041
RT-ACT2	08/27/2016–02/23/2017 (180)	21.74	277.91	0.88	0.207
RT-ACT3	08/27/2016–09/02/2016 (6)	74.83	224.50	1.0	0.268
RT-ACT4	10/10/2016–02/15/2017 (128)	4.07	272.00	0.20	0.019
RT-ACT5	10/24/2016–05/30/2017 (218)	6.48	100.93	0.50	0.005
RT-ACT6	05/02/2017–06/15/2017 (44)	4.69	49.25	0.36	0.023
RT-ACT7	08/17/2017–9/12/2017 (26)	45.69	198.00	0.86	0.236
RT-ACT8	08/25/2017–09/09/2017 (15)	91.07	273.20	1.0	0.338
RT-ACT9	9/29/2017–06/25/2018 (289)	9.81	203.00	0.32	0.043
RT-ACT10	10/06/2017–10/22/2017 (16)	55.19	294.33	0.75	0.052
RT-ACT11	10/10/2017–04/03/2018 (175)	4.73	118.43	0.35	0.016
RT-ACT12	05/21/2018–06/02/2018 (12)	17.08	68.33	1.0	0.371

### 2.5.1. Future Directions and Limitations

Validation studies, such as this, are prerequisite to applied investigations that can use these techniques to improve upon hypothesis testing in field studies with secretive or difficult-to-monitor focal taxa. This pilot study involved validation of internal implantation and field deployment procedures for ACTs with an entirely new focal group (snakes) for bio-logging studies. To the best of our knowledge, protocols for internal implantation of ACTs for long-term field monitoring have only been developed for free-living teleost fish (Wright et al. 2014; Broell et al. 2016). Implantation was essential for our study to enable long-term field deployment and to minimize the effects of the device on individual behavior. The ability to securely suture ACTs to a rib (in the same position and orientation across individuals) also further minimized inter-individual variation in ACT signals. The well-established protocols for the internal implantation of similarly sized radio transmitters in snakes combined with the relatively small size of our coupled RT-ACT implants (see image in Fig. 2.1) makes this technique widely applicable for a diversity of similarly sized snakes and other small species amenable to implantation procedures. In fact, the primary contributor to the overall size of RT-ACTs was the ACT battery, and ongoing improvements to miniaturized battery technology should continue to diminish this constraint.

The activity patterns derived from long-term ACT monitoring of *C. atrox* activity further highlight the sedentary, low-energy specialization of these cryptic sit-and-wait predators. Considerable inter- and intra-individual variation in activity was documented by constructing daily activity budgets, in some cases revealing bouts of almost total inactivity for several consecutive days (Fig. 2.3). In general, a single diel activity pattern was conserved across all

portions of the active season (spring, summer, fall), with increased activity durations occurring during the evening and nocturnal diel periods relative to the morning and diurnal periods. Activity duration across all diel periods also increased during the summer mating season for *C. atrox* on IMRS, possibly indicating elevated activity in mate-searching efforts. While these results demonstrate that pitvipers (Viperidae: Crotalinae) are intriguing model organisms for more applied extensions of our RT-ACT framework, they also carry unique challenges. This study made use of a relatively simple classification scheme targeting only two coarse behavioral modes (active vs. inactive). The highly secretive life histories of *C. atrox* made field-validation procedures more difficult than anticipated, and ultimately precluded the inclusion of additional cryptic behaviors that were infrequently or never observed in the field (i.e., feeding, mating, and male-male combat). Future studies with independent captive-validation observations would likely be required to achieve finer-scale behavioral segmentation. However, it is important to consider that some of these fine-scale behaviors will potentially require a higher ACT monitoring frequency for accurate classification, which amplifies the trade-offs between recording frequency, battery capacity, and field deployment duration. The unique biomechanics of pitvipers also largely lack the frequent and repetitive movements that aid in the segmentation of many behaviors for animals with limbs, wings, or aquatic habits, and the optimal site of internal implantation is in the lower third of the body where anterior movements are less detectable (such as striking, male-male combat, and chemosensory probing [Barbour and Clark 2012]). Any additional behaviors added to a classification scheme must be consistently distinct from other modes, and this is challenging for animals in which similar body movements are seen across behaviors (such as snakes). All of these factors combine to complicate classifying multiple discrete behaviors in *C. atrox* and other pitvipers. However, as demonstrated herein,



high model accuracy and precision are attainable given extensive “ground-truthing” data for model training, and recent work has also shown that fine-scale behavioral segmentation is achievable at low monitoring frequencies (i.e., 1-Hz) (Tatler et al. 2018). Further, recently developed complex classification algorithms can combine the most useful features from multiple models that are best suited to segment behavior within specific datasets (Ladds et al. 2017; Wilson et al. 2018). It remains to be seen if a combination of independent captive observation experiments, extensive field-validation observations, and increasingly advanced machine learning techniques can allow fine-scale behavioral segmentation under long-term, low-frequency monitoring in snakes and other small, secretive animals.

An additional limitation of the ACTs deployed for this study was the lack of a data transmitting capability, requiring re-collection of the snake at the end of field monitoring for physical extraction of the logger for manual data-offloading. This design carries the potential for total data loss if the logger is not retrieved, which occurred on several occasions during this study because of RT failure. Periodic data offloading via remote transmission to a “base station” in the field would minimize this risk (Technosmart Europe srl.) but can be cost-prohibitive if monitored individuals are dispersed across a large area (requiring multiple base stations). Lastly, statistical inference was limited with these activity data because of limited overlap in ACT sampling periods between snakes, largely as a result of premature battery failures in concurrently deployed ACTs. Moving forward, larger samples with improved data overlap will be necessary to realize the full potential of this technology in field studies with pitvipers and other focal species. Nevertheless, our validated framework illustrates the ability to remotely collect data on the timing and duration of activity at resolutions, scales, and precisions not previously possible with snakes. These data, especially when paired with additional animal-borne technologies, will

dramatically improve the ability to evaluate drivers of behavioral decisions in small-bodied and secretive terrestrial taxa inhabiting remote or inaccessible habitats. This project can also be added to a growing list of studies that demonstrate the importance of time-matched behavioral observations and cross-validation model training procedures in ACT applications (Bom et al. 2014; Wilson et al. 2015; Sur et al. 2017; Tatler et al. 2018).

## **Chapter 3: Do human-made resource hotspots and native habitats favor alternative search strategies across behavioral seasons by a desert pitviper?**

### **3.1 Abstract**

Habitat heterogeneity and local resource distribution play key roles in animal search patterns. Optimal strategies are often considered for foraging organisms, but many of the same predictions are applicable to mate searching. We monitored seasonal movement and space use by Western Diamond-backed Rattlesnakes (*Crotalus atrox*) to test whether Native Habitats (NH) and human-made Resource Hotspots (RH) facilitate alternative search strategies in response to critical resources being widely dispersed in NH and clustered in RH. Independent of habitat category, seasonal patterns largely reflected those expected under a male search-based mating system. However, divergent approaches were detected for individuals in NH and RH. NH males had larger home ranges than RH males within both behavioral seasons, and NH males increased movement distances and home ranges during the mating season while RH males displayed no seasonal shifts. NH females elevated movement distances during the mating season while RH females showed no significant seasonal shifts. Despite differing spatial tactics, habitat category had no effect on mating success or female-defense effort. Collectively, seasonal spatial patterns and observations of reproductive behavior might reflect alternative optimal strategies by NH and RH individuals, highlighting the potential for interactions among sexual selection, habitat heterogeneity, and behavioral plasticity to facilitate divergent tactics within populations.

### 3.2 Introduction

Animals move through their environments, in part, to locate resources needed for survival, such as food, water, and shelter. Patterns of movement and space use should, therefore, be shaped in response to spatiotemporal variation in the distribution of these critical resources. Movement is also costly, as it increases energy usage and exposure risks, and there is an extensive history of ecologists testing for optimization of behavior in the context of this trade-off (Emlen, 1966; MacArthur and Pianka 1966; Schoener 1971). However, numerous endogenous and exogenous factors produce variation in what might be considered optimal strategies among individuals. Habitat heterogeneity is one such factor, as variation in habitat structure directly affects the spatiotemporal arrangement of ecological resources in the environment (McIntyre and Wiens 1999; Klaassen et al. 2006; Loureiro et al. 2007). Consequently, the spatial dispersion of individuals across a landscape and their respective movement and space use patterns are strongly linked to local resource abundances.

The effects of habitat heterogeneity on movement or search strategies have been primarily investigated in foraging animals (Charnov 1976; Pyke 1984; McIntyre and Wiens 1999). However, the general predictions of optimality theory are also applicable to mate-searching behavior, as potential mating partners also represent discrete resources located through space and time (Emlen and Oring 1977). Sexual selection has frequently resulted in male movement being an effective, but costly, mechanism for locating and securing potential mates (Fromhage et al. 2016). In vertebrate mating systems that lack male parental care, female reproductive success depends primarily on access to ecological resources. Female spatial dispersion is therefore tied to the distribution of limiting ecological resources, and search

strategies by reproductive males (during mating periods) are shaped in response (Ims 1988; Duvall et al. 1993; Brown and Weatherhead 1999). Accordingly, variation in habitat quality across a heterogeneous landscape can play an important role in sex and season-specific movement and space use strategies (Shine et al. 2005). For example, if females are spatially clustered, shorter movements and reduced space use should be favored for proximate males, as the costs of moving (i.e., energy expenditure and predation risk) are minimized without sacrificing the benefits because of increased encounter rates (Emlen, 1966; MacArthur and Pianka 1966; Schoener 1971). In landscapes where female distribution might vary across different habitat patches, males are expected to adopt search strategies that reflect local conditions (Parker 1978). Behavioral plasticity, in this context, can be a critical adaptive mechanism for species in modified environments containing novel patches of resource-rich or deficient habitat.

Although historically understudied in behavioral ecology (Shine and Bonnet 2003; Clark et al. 2014), snakes, and pitvipers, specifically, are intriguing model organisms for field studies of sex and season-specific movement and space use strategies (Duvall and Schuett 1997; Glaudas and Rodríguez-Robles 2011; Bauder et al. 2016). As primarily solitary organisms, there is relative ease in identifying reproductive behavior, and male movement is the primary mate location mechanism (Duvall et al. 1993; Madsen et al. 1993; Duvall and Schuett 1997). Considerable variation in reproductive strategies inferred from spatial patterns have been reported, often in relation to the timing of the mating season and spatial distribution of females (Duvall and Schuett 1997; Brown and Weatherhead 1999). For example, sexual selection seemed to favor prolonged and highly directional (i.e., linear) mate-searching movements by male prairie rattlesnakes (*Crotalus viridis*) in Wyoming, where females were widely dispersed in the

landscape (Duvall and Schuett 1997). In a study on northern water snakes (*Nerodia sipedon*), Brown and Weatherhead (1999) found that females were clustered around key habitat features (beaver dams) within ponds and selection appeared to favor male traits related to direct competition with other males, such as male body size, rather than increased movement. Conversely, in a nearby pond that lacked beaver dams, females were more widely dispersed, and males exhibited increased movement and larger home ranges relative to the other site (Brown and Weatherhead 1999). This plasticity combined with strong seasonality in motivational states makes snakes ideal for investigating relationships between behavioral season, environmental conditions, and search patterns (King 1986; Shine 2003; Shine et al. 2003; Taylor and Booth 2016).

We aimed to explore the potential proximate and ultimate drivers of search strategies by a cryptic pitviper (Western Diamond-backed Rattlesnakes, *C. atrox*) in a unique study system in the arid northern Chihuahuan Desert. Past construction of earthen tanks (ephemeral ponds) on our study site created small, discrete resource hotspots (RH) relative to native habitats (NH), following the influx and concentration of moisture and vegetation. We exploit this human-made variation in habitat structure and quality to test whether differences in local resource distribution drive divergent seasonal movement and space use strategies among individuals within this population. We expected overall seasonal patterns to reflect a male search-based polygynandrous mating system (Rivas and Burghardt 2005), with males increasing movement and space use during the mating season in mate-searching efforts, while females show no seasonal differences. As in most taxa, female snakes have historically been characterized as passive players in mate location, and often display no seasonal differences in movement behavior (Waldron et al. 2006; Carfagno and Weatherhead 2008). However, possible cryptic female choice (i.e., females

selecting and/or rejecting potential male mating partners), benefits of multiple mating (i.e., sperm storage, sperm competition, and multiple paternity), and pheromone signaling implicate a potentially larger role than previously considered (Duvall et al. 1993; Clark et al. 2014; Shine 2003). When considering habitat category, we expected differences in local resource abundance to facilitate divergent spatial patterns. Independent of season, rattlesnakes in RH should display reduced movement and space use relative to NH counterparts. In response to the local distribution of limiting ecological resources, RH females were expected to be spatially clustered while NH females were expected to be more widely dispersed. We, therefore, predicted that RH males would display minimal or no seasonal differences in movement and space use compared to NH males, which were expected to increase movement and space use during the mating season in prolonged mate-searching efforts. We integrate movement and space use data with observations of mating and courtship behavior to further evaluate the effects of habitat heterogeneity on reproductive strategies in this unique system.

### **3.3 Methods**

#### **3.3.1 Study Site and Species**

Field data collection occurred at the 16,000 Ha Indio Mountains Research Station (IMRS; centered on 30.75°N, 105.00°W), a University of Texas at El Paso controlled property within the northern Chihuahuan Desert in Hudspeth County, Texas. Detailed descriptions of IMRS can be found elsewhere (Mata-Silva et al. 2018; Worthington et al. 2019), as we provide only a summary of relevant information. Mean annual precipitation is 235 mm, with most rainfall

(≈70%) occurring during late summer (July–September). During the active season for *C. atrox* (April–October) within the study period (June 2015–August 2018), mean daily temperature was 25.5°C, with a mean daily maximum and minimum of 32.3°C and 18.4°C, respectively.

Western Diamond-backed Rattlesnakes (*C. atrox*) are large bodied pitvipers (Serpentes: Viperidae) distributed throughout the southwestern United States into northern and central Mexico (Campbell and Lamar 2004). As one of the most widespread and abundant rattlesnakes across its geographic range, *C. atrox* has been the subject of numerous ecological investigations (Beaupre et al. 1998; Schuett et al. 2011, 2013a, 2013b), although a relative paucity of literature exists for the species in the Chihuahuan Desert. Like most pitvipers (Crotalinae), *C. atrox* are primarily sit-and-wait (i.e., ambush) predators, with rodents and lagomorphs comprising the vast majority of their diet (Beavers 1976; Spencer 2003). Considerable geographic variation in average adult body size is observed across their range (Amarello et al. 2010), but male-biased sexual size dimorphism is always maintained. Phenological studies of *C. atrox* behavior have shown that they engage in two discrete annual mating seasons, initially during late summer and again in spring after egress from winter shelters (Tinkle 1962; Taylor and Denardo 2005). However, over 30 years of field work on IMRS, reproductive behavior has only been observed from late summer through fall. We therefore considered the annual mating season for *C. atrox* on IMRS to be restricted to a single three-month period from August through October. The mating system for *C. atrox* in Arizona was recently described as “attendant polygynandry,” as males and females often mate with multiple partners within a mating period, males act as the mate-searching sex, and extended female attendance and male-male combat were observed (Clark et al. 2014). The relatively small scale of space use and high densities of *C. atrox* on IMRS made it an ideal model organism to test for the effects of historical human manipulation of habitat on



spatial and reproductive strategies. Large earthen embankments were built in several large arroyos on IMRS to create ephemeral ponds (i.e., earthen tanks) when the property supported livestock (ca. 1950s). Only a handful of tanks still function as ephemeral water bodies and they are widely dispersed across the landscape. Outside of these features, surface water is rarely available in IMRS, with the exception of a single permanent spring.

### 3.3.2 Movement and Space Use

Radio telemetry was used to collect movement and space use data from 35 *C. atrox* (18 males, 17 females) from June 2015–August 2018. Three additional females were radio-tracked during the study period but were not included in analyses after being confirmed as gravid during telemetry monitoring. Two additional males were lost to unknown predators shortly after the onset of monitoring and they did not contribute data to analyses. Radio transmitters (Holohil Systems Ltd., Models SB-2T and SI-2T) were internally implanted in rattlesnakes (Reinert and Cundall 1982) and comprised  $\leq 5\%$  of each individual's body mass at the time of the procedure. Rattlesnakes were released at the site of capture one to three days following implantation, and relocations occurred every two to three days during the active season (April–October) and biweekly during the inactive season (November–March). Locations were recorded in Universal Transverse Mercator (UTM) with a hand-held global positioning system (Garmin: Oregon 400t). Detailed behavioral observations were made during each relocation and the habitat classification was recorded. For analysis of reproductive behavior, the number of mating partners observed for each individual (i.e., courtship and coitus observed) along with the minimum number of days spent in attendance per partner were recorded during the mating season. Movement was

quantified using a series of metrics, including movement rate (Meters Per Day, MPD), calculated as the straight-line distance between successive relocation points for an individual, Distance Per Movement (DPM), calculated as the mean straight-line distance between relocation points that were  $\geq 5\text{m}$  apart, and Minimum Movement Frequency (MMF), calculated as the number of movements ( $\geq 5\text{m}$ ) made by an individual out of  $N$  relocations in a specifically defined time period<sup>40</sup>. Directionality of movements was calculated by obtaining the bearing angle between successive locations that constituted a movement by an individual. Bearings were grouped by sex and season to calculate circular variance as a proxy for directionality (Glaudias and Rodríguez-Robles 2011). Circular variance is a number between 0 and 1; with values closer to 0 indicating more directional (i.e., linear) movements. Annual movement measures were calculated and condensed into non-mating and mating seasons for analysis.

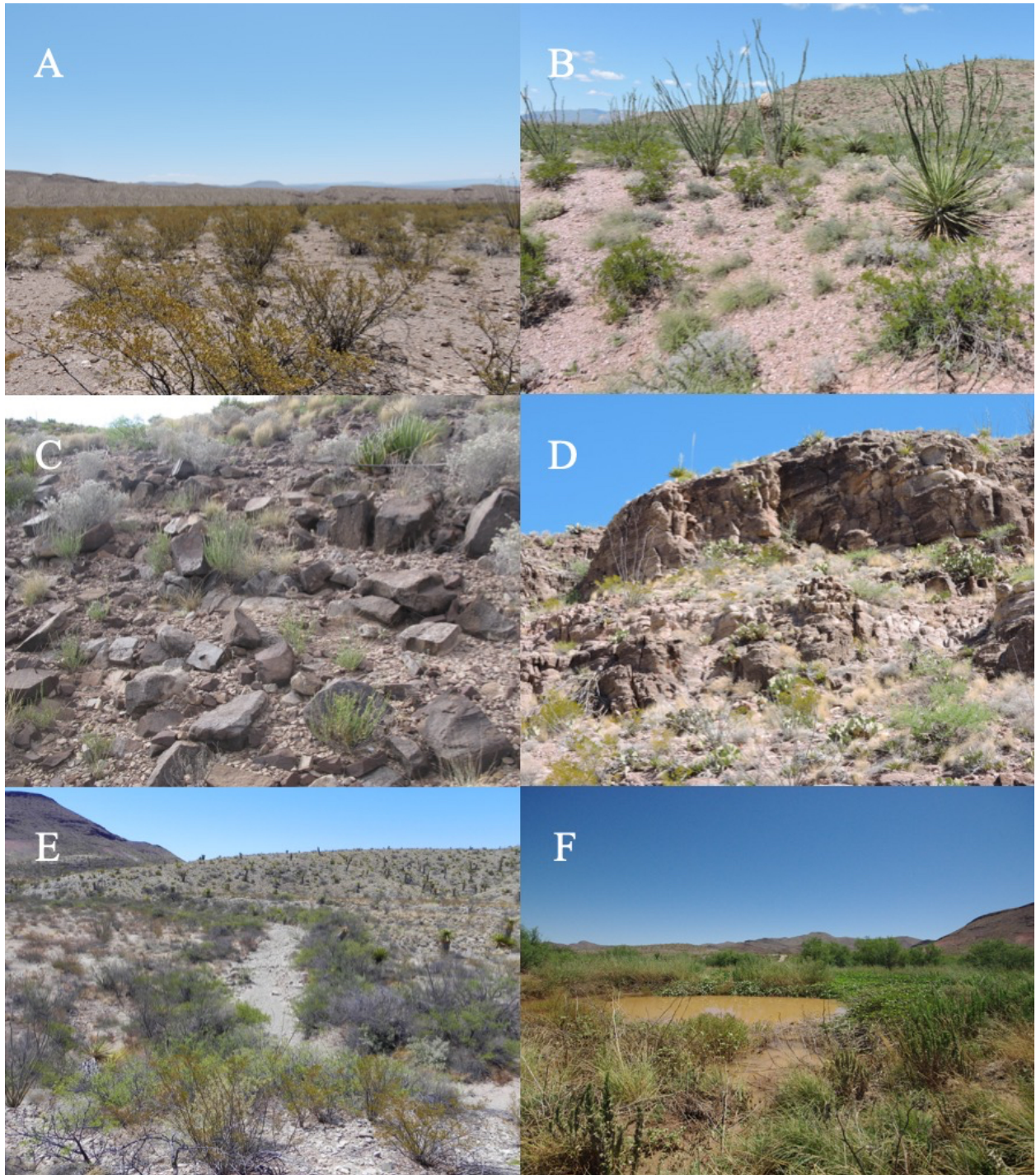
A fundamental spatial expression of an animals' series of movement steps has long been recognized as the home range (Burt 1943). This macro-scale estimation of space use allows interpretation of how individual characteristics and the external environment combine to drive spatial strategies (Börger 2008). For this study, we selected a suite of commonly presented home range estimators, including the 100% Minimum Convex Polygon (MCP) (Row and Blouin-Demers 2006), 95% fixed-kernel Utilization Distribution (UD) with the plug-in bandwidth matrix, and 50% fixed-kernel UD (core use areas) also using the plug-in bandwidth matrix (Bauder et al. 2015; Bauder et al. 2016). We selected the plug-in bandwidth matrix for UD home ranges because it was demonstrated to be robust to variation in sampling rate and duration with spatial data collected on a congener of *C. atrox* (*C. oregonus*) (Bauder et al. 2015). Seasonal home ranges were calculated only for individuals tracked across every month of a non-mating (April–July) or mating season (August–October). Movement metrics and home range sizes were

calculated in R using the *adehabitat*, *adehabitatHR*, *adehabitatLT*, and *circular* packages (Calenge 2006; R Core Team 2017).

### 3.3.3 Habitat Heterogeneity

Rattlesnakes were grouped into two habitat categories for analysis: “resource hotspot” (RH) and “native habitat” (NH). Placement into each category was determined using the proportion of relocations in each habitat classification by individuals. Habitat classifications included: alluvial flat, alluvial slope, alluvial rocky slope, rocky slope, arroyo, and earthen tank (Fig. 1). Individuals that used earthen tank habitat in greater proportions than all other habitat classes during a specified time period (i.e., year or season) were placed into the RH category. Individuals that did not meet this criterion were placed into the NH category. To test the prediction that earthen tank habitats represented relative resource hotspots for *C. atrox* on IMRS, prey abundance was estimated in each habitat class via rodent and lagomorph fecal pellet counts. Pellet counts are a validated and non-invasive method for estimating local small mammal abundance (Krebs et al. 1987; Karels et al. 2004; Glaudas and Rodríguez-Robles 2011). Counts were conducted within a m<sup>2</sup> quadrat where all visible rodent and lagomorph pellets were tallied. Ten quadrat counts were conducted at 10 m intervals along a 100 m transect through each habitat class. Transects ran at a random bearing from a UTM coordinate located at a random distance (10–100 m) and bearing from a relocation point of a radio-tracked rattlesnake located within the selected habitat class (Sperry and Taylor 2008; Haahr 2019). Pellet counts were conducted twice annually (once in April and once in August) in each habitat class during the two full years of radio telemetry data collection (2016 and 2017).

To further explore potential relationships between habitat category and the spatial patterns of proximate rattlesnakes, we calculated an index of dispersion ( $I$ ) to quantify the relative dispersion of radio-tracked rattlesnakes over time.  $I$  was calculated as the variance-to-mean ratio of the distance to the nearest neighbor for individuals at weekly intervals (i.e., every other relocation) (Glaudás and Rodríguez-Robles 2011). When  $I$  is equal to one, this indicates a random distribution, whereas values less than one and greater than one indicate a dispersed and clumped distribution pattern, respectively (Krebs 1999). This measure is not intended to provide absolute estimates of the spatial distribution of individuals in this population (which would require tracking nearly every individual in a given area), but instead may serve as a proxy for how the relative dispersion of *C. atrox* on IMRS might vary over time relative to habitat category and behavioral season.



**Figure 3.1** Representative images of each habitat classification recognized for study. A) Alluvial Flat, B) Alluvial Slope, C) Alluvial Rocky Slope, D) Rocky Slope, E) Arroyo, and F)

Earthen Tank. Native Habitat (NH) = A–E; Resource Hotspot (RH) = F. Photos taken by Vicente Mata-Silva (A–E) and DLD (F) at Indio Mounatins Research Station, Hudspeth County, Texas.

### 3.3.4 Statistical Analysis

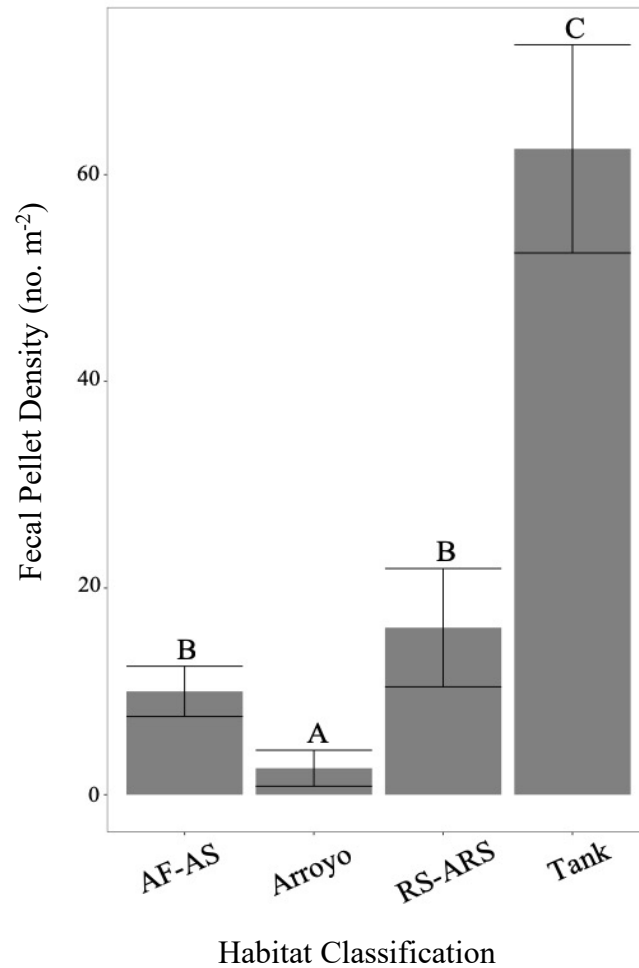
Generalized Linear Mixed-effects Models (GLMMs) within an information-theoretic framework were employed to test for the effects of sex, behavioral season, and habitat category on movement and space use patterns (Bolker et al. 2009). When dealing with non-normal and dependent data with random effects in ecology and evolutionary biology, GLMMs present several advantages over traditional linear modeling (Bolker et al. 2009), particularly the ability to model individuals and temporal designations as random effects to control for non-independence of data in time (George et al. 2015). Response variables (movement metrics and home range estimations) were modeled with separate GLMMs using the logit link. Year and individual rattlesnake within year were modeled as random effects to control for non-independence of data across time. Fixed effects included sex (male, female), behavioral season (non-mating, mating), and habitat category (RH, NH). GLMMs were also used to evaluate how reproductive behavior was associated with sex, body size (snout–vent length; SVL), movement measures (MPD, DPM, MMF, Directionality), space use estimates (100% MCP, 95% UD, 50% UD), and habitat category (RH, NH). The two response variables (number of mating partners, attendance-days-per-partner) were modeled using separate GLMMs and rattlesnake ID was included in the model as a random effect. In an exploratory analysis, appropriate transformations for each response variable were determined and employed in the model. This resulted in the following set of response variable transformations: MPD (log base 10), DPM (log base 10), MMF (Logit),

Directionality (Logit), 100% MCP (Log Base 10), 95% UD (Log Base 10), 50% UD (Log Base 10). For the Directionality and MMF response variables, beta regression models were employed, but the models using the logit link with random effects for ID provided a superior fit. Intra-class correlation coefficient (ICC) and variance were used in comparisons of model parsimony, marginal and conditional pseudo- $R^2$  measures were used to evaluate model fit, and 95% confidence intervals and  $P$ -values illustrate the effects of factor levels. Marginal pseudo- $R^2$  describes the proportion of model variance explained by fixed effects only, whereas conditional pseudo- $R^2$  describes the proportion of variance explained by both fixed and random effects. Pairwise least-squared (LS) mean interval estimates were computed using the general linear hypothesis test (GLHT) and employing multiplicity adjustments (Hothorn et al. 2008). A nonparametric ANOVA (Kruskal-Wallis) was used to test for differences in fecal pellet density among the four habitat classifications on IMRS, and Tukey's pairwise comparisons were used to evaluate differences between classes. Goodness of fit tests were used to determine if the mean dispersion ( $I$ ) of males and females grouped within habitat categories (NH and RH) differed from random. For all analyses,  $\alpha$  was set at 0.05.

### 3.4 Results

Combined (rodent and lagomorph) fecal pellet density was highest in earthen tank habitat, followed by alluvial rocky slopes and rocky slopes, alluvial slopes and flats, and arroyos (Kruskal-Wallis ANOVA:  $H_3 = 83.27$ ;  $P < 0.001$ ; Fig. 2). The higher density of a primary prey source for *C. atrox* and visibly greater vegetation density and surface water availability (Fig. 3.1)

supports our designation of earthen tanks as discrete resource hotspots relative to native habitats on IMRS.

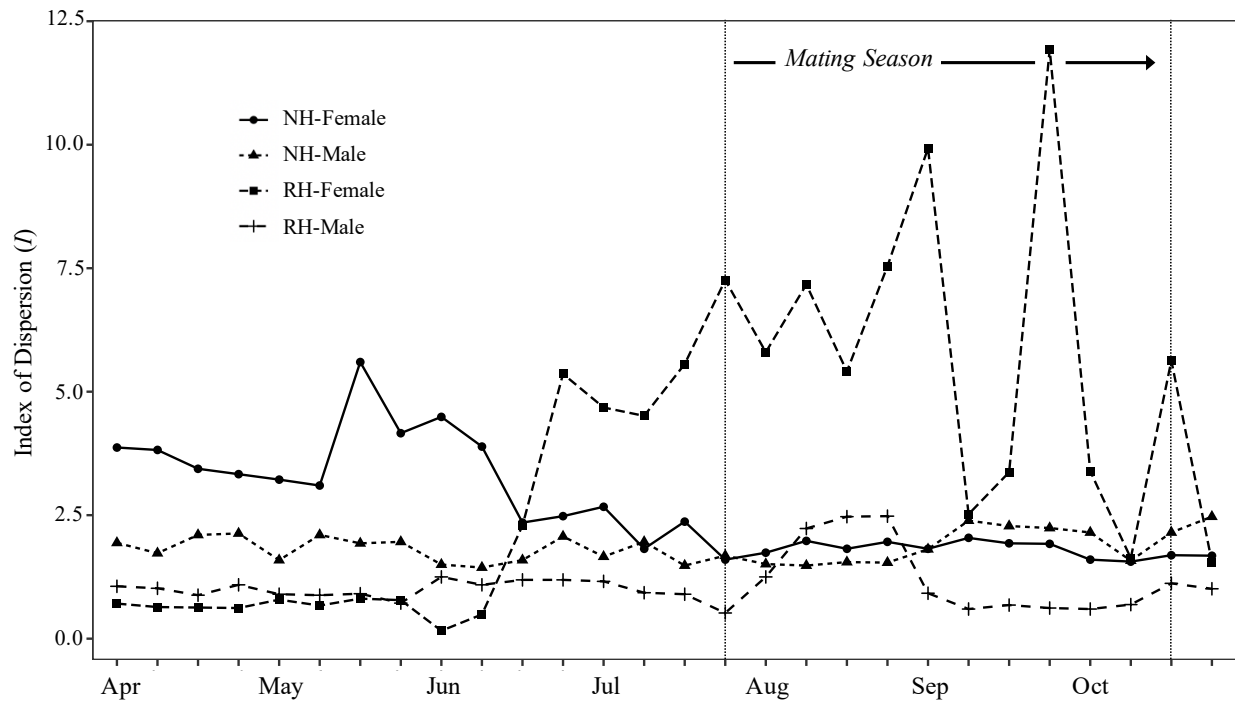


**Figure 3.2** Mean ( $\pm$  s.e.m.) rodent and lagomorph fecal pellet density (no. m<sup>-2</sup>) in habitat classifications on IMRS. Data were pooled across two sampling years (2016–2017). Classes include Alluvial Flat and Alluvial Slope (AF-AS), Arroyo, Rocky Slope and Alluvial Rocky Slope (RS-ARS), and Earthen Tank (Tank). The AF-AS and RS-ARS classes were paired for analysis because single sampling transects often intersected both habitats because of their



interconnectedness on IMRS. Letters above bars denote differences derived from post-hoc Tukey's HSD pairwise mean comparisons ( $\alpha = 0.05$ ).

Individual rattlesnakes were radio-tracked for durations ranging from 70–918 days (mean  $\pm$  s.d. =  $380 \pm 188$ ) between June 2015 and August 2018 for a cumulative total of 2,577 telemetry relocations (mean  $\pm$  s.d. =  $74 \pm 44$ ). Male ( $N=18$ ) and female ( $N=17$ ) annual movement and space use measures were calculated and condensed into behavioral seasons (non-mating, mating) and habitat categories (RH, NH). Overall (mean annual measures), males moved (mean  $\pm$  s.e.m.)  $51.06 \pm 3.9$  meters per day (MPD),  $160.33 \pm 10.5$  meters per movement (DPM), and had an MMF of  $0.76 \pm 0.03$ . Females moved  $25.89 \pm 2.2$  meters per day (MPD),  $84.07 \pm 5.7$  meters per movement (DPM), and had an MMF of  $0.69 \pm 0.03$ . Movement directionality (i.e., circular variance) was  $0.79 \pm 0.03$  for males and  $0.88 \pm 0.01$  for females. Male home range sizes averaged  $22.68 \pm 2.76$  Ha (100% MCP) and  $42.55 \pm 6.12$  Ha (95% UD), and female home ranges averaged  $4.31 \pm 0.65$  Ha (100% MCP) and  $9.88 \pm 1.78$  Ha (95% UD). Male core use area (50% UD) was  $7.35 \pm 1.46$  Ha compared to  $1.07 \pm 0.52$  Ha for females. The distributions of  $I$  were statistically different from random for all groups (RH Female, NH Female, RH Male, NH Male) (assessed at  $\alpha = 0.05$ ). All groups were generally “clustered” in space throughout the year (i.e.,  $I > 1$  for all groups), although RH females exhibited the greatest seasonal variation in  $I$  (Fig. 3.3).



**Figure 3.3** Seasonal variation in Index of Dispersion ( $I$ ) for radio-tracked *Crotalus atrox* from 2015–2018. Lines correspond to RH Female ( $N = 8$ ), NH Female ( $N = 9$ ), RH Male ( $N = 9$ ), and NH Male ( $N = 9$ ) groups. Points along individual lines are weekly variance-to-mean ratios, with values greater than one indicating a “clustered” spatial distribution and those less than one indicating a “dispersed” spatial distribution. Note the increase in spatial clustering of RH females during the mating season, whereas all other radio-tracked groups exhibit comparatively little seasonal variation in  $I$ .

Observations of reproductive behavior yielded 36 unique male-female pairings, with 14 occurring in NH and 22 in RH. Males in NH were observed with (mean  $\pm$  s.e.m.)  $0.67 \pm 0.24$  females per mating season, compared to  $1.29 \pm 0.42$  females per season for RH males. Females in NH were observed with  $1.0 \pm 0.38$  males per mating season, compared to  $1.63 \pm 0.46$  males

per season for RH females. NH males attended females for  $1.4 \pm 0.27$  days (range = 1–4) compared to  $3.2 \pm 0.97$  days (range = 1–14) for RH males. GLMM results for movement, space use, and reproductive behavior are presented below (Tables 3.1, 3.2). *F*-statistics and *P*-values are provided to illustrate the statistical significance of effects, and coefficients and 95% confidence intervals were used to further evaluate associations (Tables 3.3, 3.4).

**Table 3.1** Marginal and conditional- $R^2$ , Intraclass Correlation Coefficient (ICC-Year, ICC-ID), and variance for individual movement and space use GLMMs.

Model	Marginal- $R^2$	Conditional- $R^2$	ICC-Year	ICC-ID	Variance
MPD	0.33	0.68	0.12	0.33	0.01
DPM	0.56	0.89	0.54	0.21	0.01
MMF	0.30	0.50	0.04	0.25	1.0e-4
Directionality	0.21	0.56	0.45	1.0e-10	0.39
100% MCP	0.56	0.74	0.12	0.29	0.02
95% UD	0.50	0.76	0.52	5.5e-10	0.07
50% UD	0.42	0.89	0.45	0.35	0.25

**Table 3.2** Marginal and conditional- $R^2$ , Intraclass Correlation Coefficient (ICC-ID), and variance for individual reproductive behavior GLMMs.

Model	Marginal- $R^2$	Conditional- $R^2$	ICC-ID	Variance
Mating Partners	0.20	0.70	0.70	0.78
Female Attendance	0.51	0.64	0.69	0.89

### 3.4.1 Movement and Space Use Models

#### 3.4.1.1 Meters Per Day

There were significant main effects of sex ( $F_{1,27} = 10.08, P < 0.01$ ) and season ( $F_{1,32} = 12.12, P < 0.01$ ), along with an interaction between sex and season ( $F_{1,32} = 5.29, P < 0.01$ ). Males increased MPD during the mating season relative to the non-mating season ( $t_{43} = 3.72, P < 0.01$ ), whereas females exhibited no significant seasonal difference ( $t_{31} = 0.84, P = 0.41$ ). There was no significant main effect of habitat category upon MPD for males or females ( $F_{1,34} = 1.70, P = 0.20$ ). However, further interpreting these factors with pairwise LS mean comparisons (with multiplicity adjustments) revealed that NH males had significantly higher MPD during the mating season relative to the non-mating season ( $t_{36} = 3.12, P < 0.01$ ). Meanwhile, interval estimates from the analysis show that RH males had a smaller seasonal difference in MPD ( $t_{44} = 2.44, P = 0.05$ ). The marginal and conditional pseudo- $R^2$  measures of fit are 0.33 and 0.68, indicating a moderate level of fit.

#### 3.4.1.2 Distance Per Movement

There were significant main effects of sex ( $F_{26} = 35.74, P < 0.01$ ) and habitat ( $F_{33} = 12.14, P < 0.01$ ) with a marginal effect for season ( $F_{25} = 2.89, P = 0.10$ ). There were no significant interactions between sex, season, or habitat in the model. Pairwise GLHT comparisons revealed that both NH males and females had marginal seasonal increases in DPM

( $t_{22} = 2.32, P = 0.09$ ;  $t_{17} = 3.07, P = 0.10$ ). Conversely, there were no seasonal differences in DPM for either RH males or females ( $t_{34} = 0.29, P = 0.77$ ;  $t_{19} = -1.33, P = 0.20$ ). During the mating season there was a strong between-sex difference ( $t_{35} = 5.41, P < 0.01$ ) and, similarly, during the non-mating season there was a very similar effect ( $t_{43} = 4.66, P < 0.01$ ). Finally, DPM was significantly larger for NH males during the mating season compared to RH males ( $t_{47} = 2.08, P = 0.04$ ), and females show a similar effect ( $t_{32} = 3.19, P < 0.01$ ). However, during non-mating seasons, there was no effect of habitat for either males ( $t_{52} = 0.86, P = 0.39$ ) or females ( $t_{35} = 1.21, P = 0.23$ ), counter to the interpretation of the main effect of habitat alone. The marginal and conditional pseudo- $R^2$  measures of fit are 0.56 and 0.89, indicating a good fit.

### 3.4.1.3 Minimum Movement Frequency

There was no significant main effect of sex on MMF ( $F_{1,14} = 1.86, P = 0.20$ ), but there was a significant effect of season on MMF ( $F_{1,19} = 16.20, P < 0.01$ ) that was largely driven by males substantially increasing movement frequency during the mating season ( $t_{40} = 3.55, P < 0.01$ ). There was no association between habitat category and MMF ( $F_{1,20} = 1.96, P = 0.18$ ), but males in RH displayed a marginally greater MMF than those in NH ( $t_{45} = -1.83, P = 0.07$ ). There was a marginal three-way interaction present between sex, season, and habitat ( $F_{1,21} = 4.12, P = 0.05$ ), as males in NH, but not RH, moved more frequently during the mating season ( $t_{35} = 3.94, P < 0.01$ ), whereas females did not differ in the same manner ( $t_{29} = 0.05, P = 0.96$ ). The marginal and conditional pseudo- $R^2$  measures of fit are 0.30 and 0.50, indicating a somewhat poor level of fit.

#### 3.4.1.4 Directionality

Sex ( $F_{1,50} = 2.36$ ,  $P = 0.13$ ), season ( $F_{1,44} = 0.18$ ,  $P = 0.56$ ), and habitat category ( $F_{1,61} = 7.03$ ,  $P = 0.15$ ) did not have significant effects on movement directionality. Pairwise comparisons showed that male NH rattlesnakes made more linear movements than those in RH ( $t_{40} = 2.53$ ,  $P = 0.02$ ) indicated by the significant interaction between sex and habitat ( $F_{1,55} = 5.14$ ,  $P = 0.03$ ). However, there was no difference between RH and NH female directionality ( $t_{21} = 0.53$ ,  $P = 0.59$ ). The marginal and conditional pseudo- $R^2$  measures of fit are 0.21 and 0.56, indicating a somewhat poor level of fit.

#### 3.4.1.5 100% Minimum Convex Polygon

There was a significant main effect of sex on 100% MCPs ( $F_{1,27} = 34.33$ ,  $P < 0.01$ ), as males had larger home ranges than females ( $t_{29} = 5.75$ ,  $P < 0.01$ ). There was a significant main effect of season ( $F_{1,37} = 16.77$ ,  $P < 0.01$ ). NH Males had larger MCPs during the mating season relative to the non-mating season ( $t_{36} = 3.90$ ,  $P < 0.01$ ). There was also a significant main effect of habitat ( $F_{1,35} = 8.86$ ,  $P < 0.01$ ). Despite no significant interaction, pairwise comparisons showed that NH males had larger MCPs relative to RH males ( $t_{50} = 2.58$ ,  $P < 0.01$ ), whereas habitat had no significant effect on female MCPs ( $t_{26} = 1.50$ ,  $P = 0.15$ ). The marginal and conditional pseudo- $R^2$  measures of fit are 0.56 and 0.74, indicating a good fit.

#### 3.4.1.6 95% Utilization Distribution

There was a main effect of sex, as males had larger 95% fixed kernel UD home ranges than females ( $F_{1,21} = 28.64, P < 0.01$ ). There was also a main effect of season ( $F_{1,6} = 7.77, P = 0.03$ ) and habitat ( $F_{1,30} = 12.82, P < 0.01$ ). Males in NH increased 95% UD during the mating season relative to the non-mating season ( $t_{27} = 2.67, P = 0.01$ ), whereas RH males displayed no seasonal difference ( $t_{38} = 1.34, P = 0.19$ ). Season had no effect on female 95% UD ( $t_{22} = 1.10, P = 0.28$ ), but habitat had an effect on female 95% UD, as NH females had larger 95% UD than RH females ( $t_{19} = 12.62, P = 0.02$ ). The marginal and conditional pseudo- $R^2$  measures of fit are 0.50 and 0.76, indicating a good fit.

#### 3.4.1.7 50% Utilization Distribution

For core use area, there were main effects of sex ( $F_{1,29} = 7.29, P = 0.01$ ), season ( $F_{1,11} = 18.95, P < 0.01$ ), and habitat ( $F_{1,37} = 11.76, P < 0.01$ ). Core use areas generally followed the same pattern as 95% UD (although not as strongly), where NH males increase core use area size during the mating season ( $t_{12} = 2.02, P = 0.05$ ) and RH males displayed no seasonal difference ( $t_{19} = 1.65, P = 0.12$ ). Habitat had no effect on female core use area ( $t_{12} = 1.50, P = 0.15$ ). The marginal and conditional pseudo- $R^2$  measures of fit are 0.42 and 0.89, indicating a good fit.

**Table 3.3** Coefficients, 95% confidence intervals, and *P*-values for movement and space use model parameters. NM = Non-mating Season, RH = Resource Hotspot.

Model	Coefficient	95% Confidence Interval		P
Parameter				
Meters Per Day		Lower	Upper	
Sex (Male)	-0.36	-0.58	-0.15	< 0.01
Season (NM)	-0.28	-0.46	-0.10	< 0.01
Habitat (RH)	-0.07	-0.30	0.17	0.20
Sex*Season (NM)	0.21	-0.03	0.45	< 0.01
Sex*Habitat (RH)	-0.04	-0.40	0.30	0.97
Season*Habitat (RH)	-0.08	-0.39	0.25	0.77
Sex*Season (NM)*Habitat (RH)	0.09	-0.37	0.54	0.70
Distance Per Movement				
Sex (Male)	-0.22	-0.34	-0.09	< 0.01
Season (NM)	-0.10	-0.18	-0.02	0.10
Habitat (RH)	-0.15	-0.28	-0.02	< 0.01
Sex*Season (NM)	-0.04	-0.18	0.07	0.61
Sex*Habitat (RH)	-0.17	-0.37	0.02	0.30
Season*Habitat (RH)	0.08	-0.08	0.25	0.01
Sex*Season (NM)*Habitat (RH)	0.15	-0.07	0.36	0.22



<b>Minimum Movement</b>				
<b>Frequency</b>				
<i>Sex (Male)</i>	-0.17	-0.34	-0.01	0.19
<i>Season (NM)</i>	-0.35	-0.50	-0.19	< 0.01
<i>Habitat (RH)</i>	0.09	-0.10	0.28	0.18
<i>Sex*Season (NM)</i>	0.34	0.11	0.55	0.15
<i>Sex*Habitat (RH)</i>	0.03	-0.27	0.30	0.19
<i>Season*Habitat (RH)</i>	0.16	-0.14	0.44	0.71
<i>Sex*Season (NM)*Habitat (RH)</i>	-0.39	-0.78	0.04	0.05
<b>Directionality</b>				
<i>Sex (Male)</i>	0.58	-0.15	1.32	0.13
<i>Season (NM)</i>	-0.46	-1.05	0.16	0.56
<i>Habitat (RH)</i>	0.61	-0.18	1.40	0.15
<i>Sex*Season (NM)</i>	0.74	-0.15	1.63	0.23
<i>Sex*Habitat (RH)</i>	-0.90	-2.04	0.25	0.03
<i>Season*Habitat (RH)</i>	0.65	-0.58	1.88	0.33
<i>Sex*Season (NM)*Habitat (RH)</i>	-0.47	-2.07	1.15	0.58
<b>100% Minimum Convex</b>				
<b>Polygon</b>				
<i>Sex (Male)</i>	-0.72	-1.02	-0.41	< 0.01
<i>Season (NM)</i>	-0.22	-0.49	0.03	< 0.01
<i>Habitat (RH)</i>	-0.30	-0.63	0.03	< 0.01

<i>Sex*Season (NM)</i>	-0.04	-0.40	0.34	0.91
<i>Sex*Habitat (RH)</i>	0.14	-0.34	0.62	0.45
<i>Season*Habitat (RH)</i>	-0.21	-0.67	0.29	0.25
<i>Sex*Season (NM)*Habitat (RH)</i>	0.04	-0.62	0.65	0.90
<b>95% Utilization Distribution</b>				
<i>Sex (Male)</i>	-0.63	-0.92	-0.31	< 0.01
<i>Season (NM)</i>	-0.37	-0.60	-0.12	0.03
<i>Habitat (RH)</i>	-0.35	-0.66	-0.03	< 0.01
<i>Sex*Season (NM)</i>	0.24	-0.12	0.63	0.28
<i>Sex*Habitat (RH)</i>	-0.07	-0.63	0.48	0.62
<i>Season*Habitat (RH)</i>	0.07	-0.39	0.53	0.86
<i>Sex*Season (NM)*Habitat (RH)</i>	-0.07	-0.99	0.74	0.85
<b>50% Utilization Distribution</b>				
<i>Sex (Male)</i>	-0.89	-1.47	-0.26	0.01
<i>Season (NM)</i>	-0.55	-0.89	-0.19	< 0.01
<i>Habitat (RH)</i>	-0.69	-1.21	-0.12	< 0.01
<i>Sex*Season (NM)</i>	0.05	-0.47	0.56	0.39
<i>Sex*Habitat (RH)</i>	0.19	-0.95	1.09	0.40
<i>Season*Habitat (RH)</i>	-0.53	-1.23	0.14	0.27
<i>Sex*Season (NM)*Habitat (RH)</i>	0.41	-0.75	1.65	0.49

### 3.4.2 Reproductive Behavior Models

There was a significant main effect detected for SVL upon attendance-days-per-partner ( $t_{13} = 2.93$ ,  $P = 0.01$ ), as both male and female SVL was positively related to number of days spent in attendance. SVL did not have a significant effect on the number of observed partners ( $t_{14} = 1.25$ ,  $P = 0.17$ ). Habitat category and all movement and space use metrics had no significant effect on reproductive behavior (Table 3.2). Sex of the radio-tracked rattlesnake did not have a strong effect on the number of observed mating partners (i.e., males and females encountered a similar number of potential mating partners) or attendance-days-per-partner, and there were no significant interactions between factors (Table 3.2). The marginal and conditional pseudo- $R^2$  measures of fit are 0.20 and 0.70 for the mating-partner model, indicating a moderate level of fit, and 0.51 and 0.64 for the female-attendance model, indicating a good fit.

**Table 3.4** Coefficients, 95% confidence intervals, and *P*-values for reproductive behavior model parameters. RH = Resource Hotspot, SVL = Snout–vent Length, MPD = Meters Per Day, DPM = Distance Per Movement, MMF = Minimum Movement Frequency, MCP = Minimum Convex Polygon, UD = Utilization Distribution.

Model	Coefficient	95% Confidence Interval		P
Parameter				
Number of Mating Partners		<i>Lower</i>	<i>Upper</i>	
<i>Sex (Male)</i>	-1.56	-3.79	0.43	0.14
<i>Habitat (RH)</i>	0.49	-0.84	1.40	0.63
<i>SVL</i>	1.49	-0.21	1.53	0.16
<i>MPD</i>	-1.17	-1.08	0.27	0.28
<i>DPM</i>	1.32	-0.25	1.32	0.21
<i>MMF</i>	0.52	-0.39	0.67	0.61
<i>Directionality</i>	0.09	-0.42	0.46	0.93
<i>100% MCP</i>	0.69	-0.80	1.65	0.52
<i>95% UD</i>	-0.91	-5.51	2.01	0.39
<i>50% UD</i>	0.76	-1.84	4.20	0.47
Attendance Days Per Partner				
<i>Sex (Male)</i>	-1.76	-6.30	0.35	0.10
<i>Habitat (RH)</i>	0.77	-1.18	2.70	0.46
<i>SVL</i>	3.69	1.09	1.95	< 0.01
<i>MPD</i>	-0.08	-1.10	3.64	0.93

<i>DPM</i>	0.83	-0.82	2.05	0.42
<i>MMF</i>	-0.54	-1.26	0.72	0.60
<i>Directionality</i>	0.05	-0.86	0.91	0.96
<i>100% MCP</i>	-0.11	-2.71	2.42	0.91
<i>95% UD</i>	-1.04	-11.30	3.44	0.31
<i>50% UD</i>	1.11	-2.49	8.96	0.29

### 3.5 Discussion

For vertebrate mating systems without male parental care, males are expected to make the largest investment in mate-searching (Emlen and Oring 1977; Parker 1984). Among snakes, this is often displayed through a seasonal increase in movement and space use by males (Shine 2003), but intrinsic sexual differences in spatial ecology have also been observed outside of mating periods (Glaudas and Rodríguez-Robles 2011). Therefore, we expected male *C. atrox* to display greater movement and space use than females across both the non-mating and mating seasons, along with males elevating movement and space use during the mating season relative to the non-mating season, whereas females would display no seasonal shifts. We also expected males to make more linear movements compared to females, and to increase directionality during the mating season in efforts to efficiently locate females.

As predicted, male movement distances (MPD, DPM) and all space use estimates (100% MCP, 95% UD, 50% UD) are greater than female measures across seasons, but there are no differences between male and female MMF and Directionality. Males also exhibit the characteristic increase in all movement (*sans* Directionality) and space use measures during the

mating season relative to the non-mating season. The lack of an increase in movement directionality by males during the mating season could indicate that targeted resources (i.e., prey, mating partners) are spatially dispersed similarly across seasons, but this is not supported by the seasonal differences observed for other spatial measures. Unexpectedly, females increase DPM during the mating season, whereas there is no seasonal difference for other movement and space use metrics. Females can participate in mate location if the investment is low relative to the potential gain (Emlen and Oring 1977; Fromhage et al. 2016). One hypothesis of female participation is the mate facilitation model, whereby females increase movement to improve detection by males via lipid-based pheromone signaling (Blouin-Demers and Weatherhead 2002; Jellen and Aldridge 2014). It is possible that the seasonal increase in female DPM reflects an active role in mate location, although we are unable to completely rule out other potential drivers, such as foraging behavior (female *C. atrox* occasionally forage across behavioral seasons on IMRS) and weather conditions. However, several additional factors support the mate facilitation model as an explanation for this pattern. Because of the large energy investment made by female snakes in reproduction, annual parturition is often rare (Aldridge and Duvall 2002; Shine 2003), but female *C. atrox* can still benefit from mating annually because of long-term sperm storage (although this could also reduce the need for annual copulation), sperm competition, and multiple paternity (Clark et al. 2014). This appears to be the case at IMRS, as females underwent parturition biennially or less frequently, but were observed copulating with males annually. Therefore, we are confident that the patterns of female movement reported here are representative of reproductive individuals.

Organisms have the ability to alter behavior in response to environmental changes, and for searching individuals, habitat heterogeneity and the consequent spatial distribution of

resources is fundamental to understanding both foraging (Charnov 1976) and reproductive strategies (Emlen and Oring 1977). On IMRS, earthen tanks function as relative resource hotspots for *C. atrox* given the increased abundance of moisture, shelter (vegetation), and prey (rodents and lagomorphs; Fig. 2). When resources are clustered in space, optimality theory predicts that shorter movements and reduced space use should be favored, as the various costs of moving (i.e., energy expenditure and predation risk) are minimized without sacrificing the benefits given increased encounter rates (Emlen 1966; MacArthur and Pianka 1966; Schoener 1971). Therefore, we hypothesized that overall (i.e., independent of season), NH rattlesnakes would display greater movement and space use relative to RH rattlesnakes. Independent of behavioral season, these predictions are only partially supported. NH males have substantially larger home range sizes (100% MCP, 95% UD, 50% UD) than RH males, and NH females have significantly larger 95% UD home ranges than RH females. There are no within-sex differences in mean annual movement measures (MPD, DPM, MMF) between NH and RH rattlesnakes, but NH males did make more linear movements than RH males. When accounting for season, the effects of habitat category become more apparent, and two distinct patterns emerge (Fig. 3.4). NH males substantially increase all movement (*sans* Directionality) and space use measures during the mating season relative to the non-mating season, whereas RH males display no seasonal differences characteristic of prolonged mate-searching behavior. The dichotomy between NH and RH male home range sizes is particularly notable, both in terms of the magnitude of the increase during the mating season by NH males and the relative difference between NH and RH males within seasons (Fig. 3.4). We suspect that these divergent seasonal patterns by NH and RH males reflect a relative difference in the cost-benefit trade-off associated with moving in each habitat type. As with other critical resources, if potential mating partners are

spatially consolidated into discrete sites or patches in the environment, reduced movement and space use are favored (Polis et al. 1998; Muniz et al. 2018). If reproductive females are spatially clustered around RHs (Fig. 3.3) in response to a similar clustering of ecological resources, the spatial and reproductive strategies of proximate males should be shaped accordingly during the mating season (Bradbury et al. 1986; Brown and Weatherhead 1999). This seems to hold true for our study, as the seasonal shift in motivational state (i.e., foraging vs. mating) does not accompany a shift in search strategy by RH males. Conversely, NH males make longer and more frequent movements within larger home ranges during the mating season relative to the non-mating season, presumably in search of more widely dispersed females.

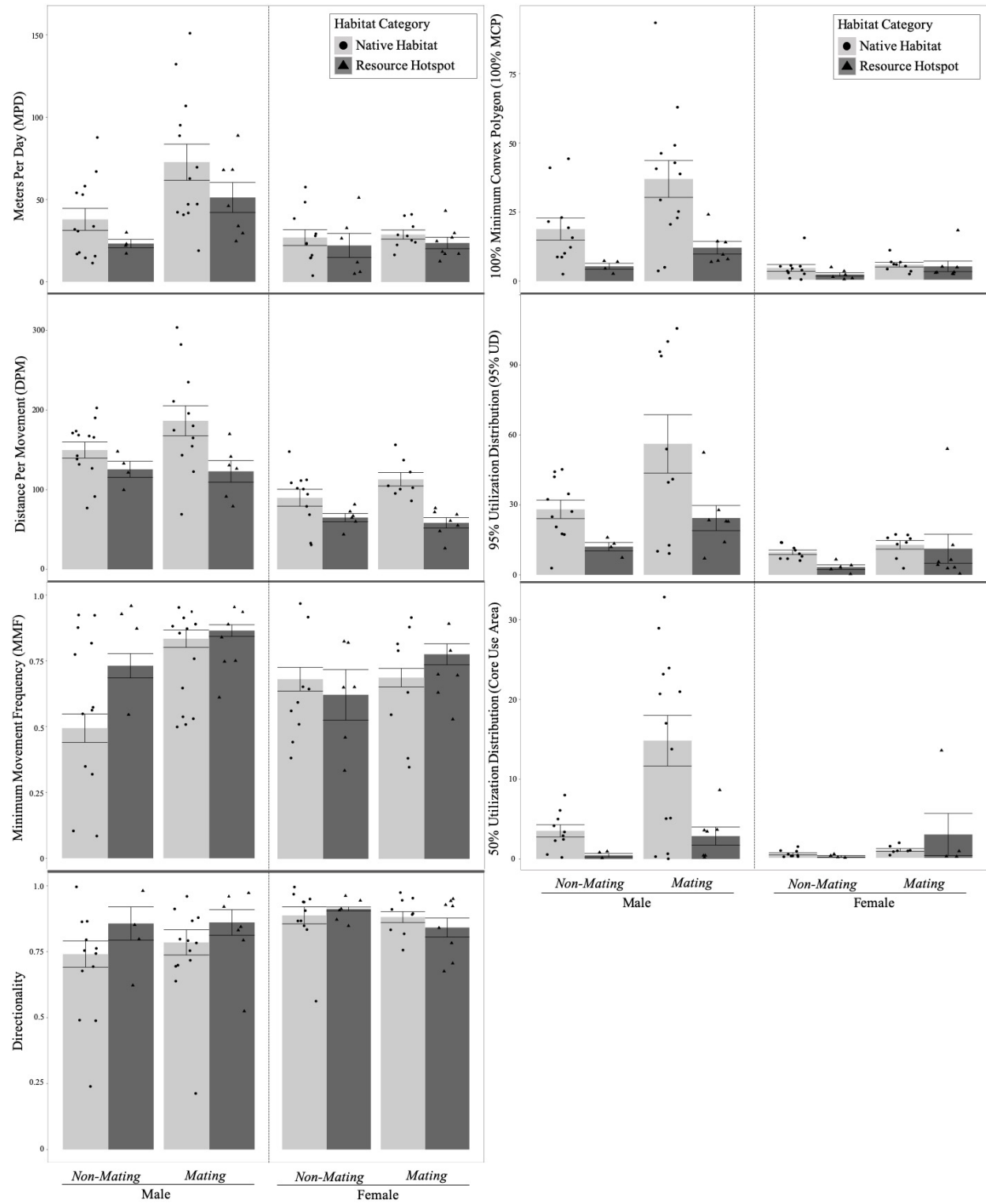
The home range size of NH females (mean  $\pm$  s.e.m.; 100% MCP:  $3.28 \pm 1.16$  Ha; 95% UD:  $8.18 \pm 3.92$  Ha) is comparable to the spatial extent of earthen tanks on IMRS (three tanks represented during this study covered 0.52, 1.04, and 1.20 Ha), and therefore, our ability to detect differences in movement and space use between NH and RH females is limited. Consequently, the effects of habitat category on female seasonal spatial patterns are dampened relative to males (Fig. 3.4). However, independent of season, 95% UD home ranges were also significantly greater for NH females relative to RH females. LS mean comparisons with multiplicity adjustments also show that the main effect of season on female DPM is driven primarily by NH females, as they elevate DPM during the mating season, whereas RH females display no seasonal difference. As discussed previously, the NH female elevation of DPM during the mating season might represent mate location efforts via advertising movements to enhance detection by males. The increase in DPM by NH females could be in response to males being widely dispersed in NH, making females that afford larger spatial investments in pheromone signaling more easily detectable (Blouin-Demers and Weatherhead 2002). While there was not a



significant seasonal difference, RH females marginally increase in MMF during the mating season (Fig. 3.4), which could reflect a similar tactic, but at a reduced spatial scale, as elevating the frequency of short-distance movements within RH might carry the benefits of improved male detection while minimizing exposure costs. However, increased movement could also reflect an increased need for food acquisition (Glaudas and Alexander 2017), particularly in the case of females which undergo vitellogenesis following the mating season (Schuett et al. 2011). Female home-range sizes within habitat categories showed no seasonal differences. Increasing movement within home ranges, but not home ranges overall, has been observed for other female snakes during mating periods (Glaudas and Rodríguez-Robles 2011; Jellen and Aldridge 2014).

Alternative reproductive strategies have been reported for a wide variety of vertebrate and invertebrate taxa, often in cases where different approaches are related to variation in male phenotype (Gross 1996; Shine et al. 2003; Immler et al. 2004). Another driver of alternative mate location and procurement tactics is variation in the spatiotemporal distribution of females (Emlen and Oring 1977; Shine et al. 2005). Male strategies tailored to female distribution have been reported for several rattlesnake species. Prairie Rattlesnakes (*C. viridis*) exhibited prolonged mate-searching without attendance in a population where females were widely dispersed during the late-summer mating season (Duvall and Schuett 1997). Speckled Rattlesnakes (*C. pyrrhus*) displayed an intermediate strategy where males increased movement in search of females but also engaged in attendance. This approach could have been a response to females being aggregated around den sites (i.e., communal winter shelters) during the spring mating season but still competitively limited because of a male-biased operational sex ratio (Glaudas and Rodríguez-Robles 2011). An example of variation in male strategies within a population was reported for Timber Rattlesnakes (*C. horridus*), where males showed extensive attendance

behavior when females were clumped around rock outcrops early in the mating season, but later shifted to prolonged mate-searching when females dispersed away from these features (McGowan and Madison 2008). A key feature conserved across all of these congeneric examples is the link between the spatial distribution of specific habitats or limiting ecological resources and the spatial distribution of females and, consequently, male mate-searching strategies during the mating season.



**Figure 3.4** Mean ( $\pm$  s.e.m.) male and female *Crotalus atrox* movement and space use measures within behavioral season (Non-mating, Mating) and habitat category (Native Habitat, Resource Hotspot), pooled across all sampling years (2015–2018). Movement measures include Meters-Per-Day (top left), Distance-Per-Movement (middle left), Minimum Movement Frequency (middle left), and Directionality (bottom left). Space use estimates include 100% Minimum Convex Polygon home range (100% MCP) (top right), 95% fixed-kernel Utilization Distribution home range (95% UD) (middle right), and 50% fixed-kernel Utilization Distribution core use area (50% UD) (bottom right).

Broadly, the mating system of *C. atrox* on IMRS can be characterized as an intermediate between prolonged mate-search and female-defense polygynandry, as both males and females often mate with multiple partners within a single mating season, males make a substantial investment in mate-searching, and males occasionally exhibit extended female attendance. However, the divergent movement and space use patterns exhibited by NH and RH rattlesnakes might be indicative of two alternative strategies within the broader intermediate system. The substantial increase in movement and space use by NH males during the mating season is characteristic of prolonged mate-searching polygynandry, wherein male movement is expected to be the primary determinant of mate location and mating success. Although the reproductive behavior GLMMs show no strong relationships between NH male movement or space use and the number of observed mating partners, this could be an artifact of our sampling protocols, given the relatively small sample of radio-tracked males in NH that contributed reproductive data ( $N = 9$ ) and that the number of mating partners for individual NH males ranges only from zero to two. A more compelling indication of the effectiveness of the NH male search strategy is that

there is no significant difference in the number of observed mating partners between NH ( $0.67 \pm 0.24$ ) and RH males ( $1.29 \pm 0.42$ ), despite their contrasting seasonal search patterns. The number of mating partners also do not differ significantly between NH ( $1.0 \pm 0.38$ ) and RH females ( $1.63 \pm 0.46$ ) (Table 3.2).

A narrow-sense mating system classified as “hotspot polygyny” can occur when females cluster within a small area because of a similar clustering of limited ecological resources (Duvall et al. 1993). The index of dispersion ( $I$ ) illustrates that radio-tracked RH females exhibit a highly clustered distribution during the mating season (Fig. 3.3), whereas other groups show relatively little seasonal variation in  $I$ . Such a clustering of females in RH could potentially intensify male-male competition, and elevated female-defense behavior by males within a female-hotspot might be favored over prolonged-mate searching. In the case of RH males, this is partially corroborated by the lack of any increases in movement or space use during the mating season, and the lack of associations between RH male movement and number of mating partners. Although there is no significant difference in attendance-days-per-partner between RH and NH males (Table 3.2), RH males attended females for  $3.2 \pm 0.97$  days compared to  $1.4 \pm 0.27$  days by NH males.

Alternatively, within a hotspot, multiple mating by females (observed on IMRS), multiple paternity of litters (reported elsewhere for *C. atrox* [Clark et al. 2017]), and a relative abundance of females, could potentially combine to narrow the typically male-biased operational sex ratio (OSR) and reduce the force of sexual selection on female-defense behavior (Duvall et al. 1993). Ultimately, the measures of dispersion ( $I$ ) reported here are derived from relatively small samples and are not a reliable substitute for rattlesnake densities within NH and RH. Estimates of local densities are necessary for a detailed assessment of habitat-specific OSR, which is key to linking reproductive strategies to local conditions.

Strategic movement patterns have been widely studied in foraging organisms, but less frequently in mate-searching behavior. Our study system offered a unique opportunity to test how variation in local resource distribution influences movement and space use by a cryptic predator across behavioral seasons. In general, sex and season-specific patterns reflect those expected under a male search-based polygynandrous mating system, as males move greater distances and use more space than females, and males substantially increase movement and space use during the mating season. However, by accounting for rattlesnakes using earthen tank habitats and those using native habitats, we document divergent seasonal search patterns relative to habitat category. NH males display greater space use than RH males, and NH males significantly increase movement and space use during the mating season, whereas RH males show no significant seasonal shifts. Despite this, the number of observed mating partners for NH and RH males does not differ, indicating that the opposing spatial patterns might represent alternative optimal male reproductive strategies. We also found evidence that females might play a less prominent, but active role in mate location by slightly elevating movement during the mating season to augment detection by searching males, although additional factors (foraging behavior, weather) could contribute to this pattern. Similar to males, habitat category appears to favor alternative seasonal strategies for females, as NH females increase movement distances during the mating season, whereas RH females show no significant seasonal shifts. The number of mating partners for females also did not differ relative to habitat category. Seasonal patterns of movement and space use combined with detailed observations of reproductive behavior provide evidence for two distinct narrow-sense mating systems classifications on IMRS: prolonged mate-searching polygynandry for NH *C. atrox*, and a form of female-hotspot polygynandry for RH *C. atrox*, highlighting the potential for multiple interacting mechanisms (i.e., sexual selection,

habitat heterogeneity, behavioral plasticity) to drive divergent spatial strategies within populations, and further demonstrating the dynamic role of ecological factors in the evolution of animal mating systems. These results have also effectively set the stage for future investigations into whether differences in OSR at fine spatial scales (related to possible variation in *C. atrox* densities between NH and RH) can further elucidate the link between reproductive strategies and resource distribution in this system.

## **Chapter 4: Conclusions**

### **4.1 Implications for Studies of Snake Movement Ecology**

Snakes are historically understudied in ecology, in large-part because of their highly secretive life histories (Dorcas and Willson 2009; Durso et al. 2011). While hand-held radio telemetry, alone, proved to be transformative for field studies (Reinert 1992), considerable efforts are underway to incorporate additional bio-logging technologies with these unique model organisms (Chapter 2; reviewed in Beaupre 2016). However, most of the popular sensors seen in animal movement and bio-logging research are not currently applicable for use in snakes, largely because of periodic ecdysis that prevents long-term external attachment and bulky sensors or batteries that prohibit internal implantation procedures in most species (Beaupre 2016). This dissertation capitalizes on recent miniaturization of solid-state sensor technologies to demonstrate the value of a framework that combines novel (accelerometer) and traditional (radio telemetry) technologies for studying the movement behavior of wild snakes by integrating continuous activity data (recorded over extended durations) and long-term spatial data. However, while ACTs effectively circumvent many of the traditional limitations imposed by hand-held radio telemetry, it is important to recognize the inherent limitations associated with accelerometry in snakes and other small-bodied species.

Although Chapter 2 focusses on the advantages accelerometry offers over traditional data collection techniques in snake movement ecology, Chapter 3 highlights the retained value of hand-held radio telemetry. Given the unique challenges imposed by snakes, radio telemetry will



undoubtedly remain a valuable tool for snake ecologists moving forward. Even in novel bio-logging applications, radio transmitters will necessarily be paired with any novel sensor applied to snakes to ensure retrieval of implants at the end of field monitoring. Furthermore, a method to replace telemetry as a tool to collect spatial data from a longitudinal sample of snakes has yet to emerge. Global Positioning System (GPS) loggers, for instance, have been revolutionary for spatial studies with animals (Hebblewhite and Haydon 2010; Wilmers et al. 2015), but their use in snakes, at present, is severely limited by the need for an external antenna to acquire a satellite signal. In addition to the previously discussed problems associated with external attachment on snakes, most species (especially in arid systems) tend to spend extended periods in underground refugia, which effectively blocks satellite signals. For pitvipers, specifically, the often-sedentary nature of individuals also limits the benefits of high-resolution spatial data and brings into question whether the increased “tag-load” on individuals is worth the data returned (Wilson et al. 2019). For these reasons, radio telemetry remains the best available tool for estimating spatial metrics in snakes, whereas ACTs offer a novel and greatly improved method for monitoring fine-scale activity patterns (i.e., timing and duration of movement). Ultimately, our results demonstrate that data provided by accelerometry and radio telemetry are complementary, simultaneously informing methods for different aspects of movement ecology. When paired, RT-ACTs provide exciting avenues for future research on the temporal and spatial dimensions of snake movement and behavior.

## 4.2 RT-ACT Framework: Future Directions

Studying animals in their natural environments without disturbing their normal behavior has been an enduring and central challenge in ecology. The “golden-age of animal bio-logging” continues to revolutionize the way researchers study wild animals by circumventing nearly all of the traditional observational constraints (Wilmers et al. 2015). The rich tool kit provided by these technologies can offer important and often surprising insights in both descriptive and applied studies. However, among the hundreds of bio-logging applications found in the primary literature, the vast majority are used in marine systems or with avian focal taxa (Brown et al. 2013). This taxonomic bias is not surprising, considering that these organisms have historically been the most difficult to observe and study in nature, but the exciting potential of bio-logging technologies extends to nearly every ecosystem on Earth (Chmura et al. 2018). To realize the full potential of these “techno-ecological” approaches, particularly for informing conservation objectives and management strategies, researchers must capitalize on the ongoing advancement and miniaturization of sensors to develop and validate animal-borne bio-logging protocols for a broader diversity of study organisms (Allan et al. 2018). The integrative framework developed and validated in Chapter 2 is an important contribution to this end. Below, I discuss some of the broader implications of this research, explore avenues for future study, and comment on prominent challenges for future bio-logging studies using the RT-ACT framework.

The urgency to develop and test innovative methods for field studies on the interactions between animal behavior and internal and external factors is strengthened in the face of ongoing global climate change (Nathan et al. 2008; Wilson et al. 2015). Among the many species affected by altered climate conditions, ectotherms are considered to be particularly at-risk in the face of

rising environmental temperatures and increased temperature variability (Deutsch et al. 2008; Paaijmans et al. 2013). However, there is potential for plasticity in phenology, behavior, distribution, and physiology to be an important mitigating mechanism for animals in the face of ongoing climate changes (Huey et al. 2012; Rabaiotti and Woodroffe 2019). One important behavioral mechanism by which an ectotherm might respond to shifting environmental conditions is by changing activity patterns. Temporal scale is a key consideration in this regard, as relatively coarse scales, such as monthly, seasonal, or annual time frames, sometimes fail to consider the effects of increased variation at finer scales. Daily variation in temperature and other climatic factors can drive significant variation in the frequency, timing, and duration of activity, and thus carry significant implications for the relative role and impact of the affected species at the ecosystem level. Moving forward, high-resolution bio-logging technologies will become increasingly important for documenting and understanding organismal responses to changes in abiotic environments (Shepard et al. 2008; Hebblewhite and Haydon 2010; Brown et al. 2013; Papastamatiou et al. 2015; Wilson et al. 2015; Pagano et al. 2017). In a review of the potential of various bio-loggers to improve studies of physiological and ecological responses to climate change, Chmura et al. (2018) noted that there is still considerable debate over whether phenotypic plasticity will be sufficient to buffer populations from ecosystem alterations. Direct observations of individual responses to variation in climate condition were previously impossible for species that had exceedingly large home ranges or inhabited remote or inaccessible environments (Chmura et al. 2018). Even when individuals can occasionally be watched directly (such as the rattlesnakes studied herein), many of the responses of interest (i.e., shifts in activity patterns, physiological responses) are cryptic and difficult or impossible to simultaneously

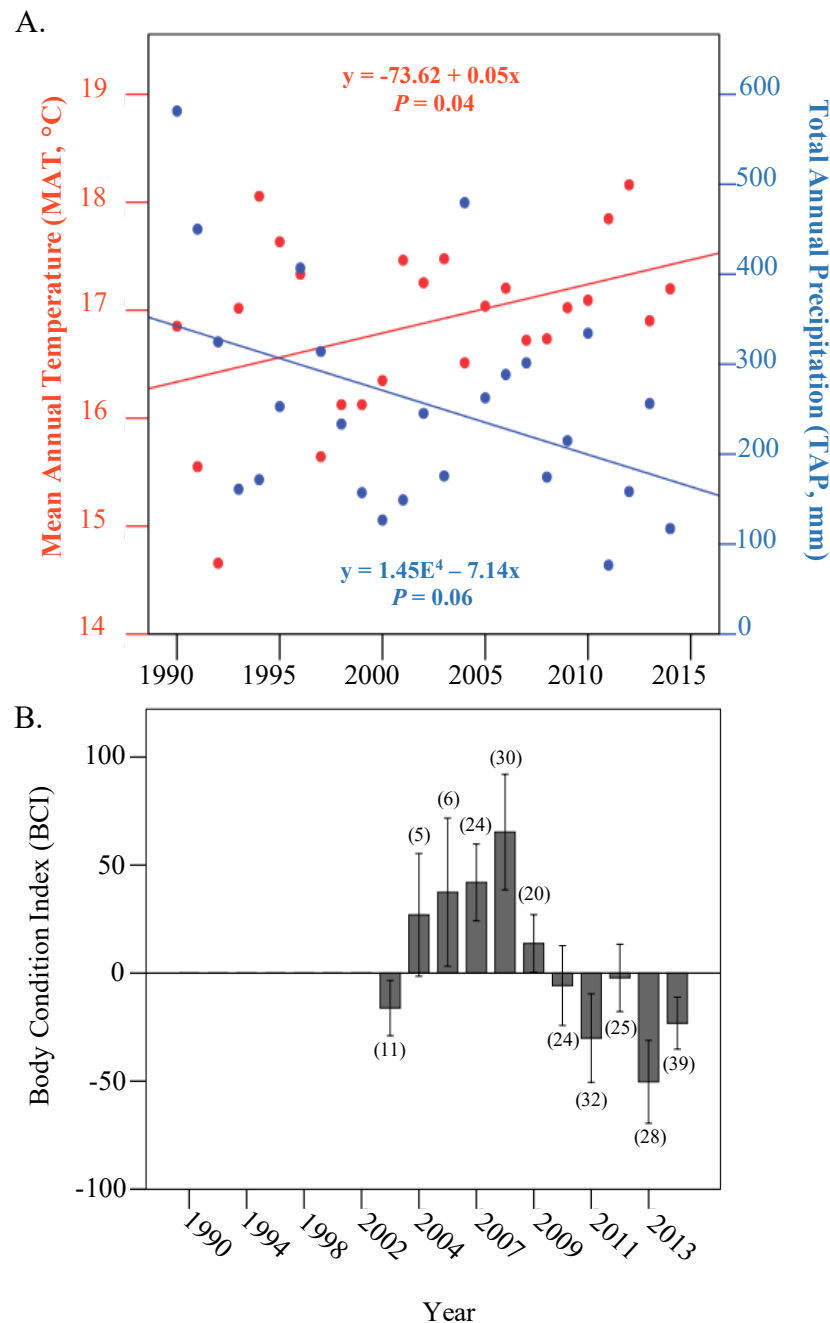
monitor across multiple individuals with traditional methods alone. ACTs effectively eliminate these constraints by enabling remote and continuous recording of activity.

Abundant ectothermic predators, including many pitvipers, seem ideally suited for studies attempting to link variation in activity patterns to environmental conditions. Snake activity is tightly coupled to environmental temperatures, increasing the potential for altered thermal regimes to affect their behavior, and, in turn, their relative impact and role at the ecosystem level (Beaupre 2009). As low-energy specialists, pitvipers and other snakes are often prominent vertebrate predators in a diverse array of ecosystems (Nowak et al. 2008). One of the few comprehensive studies on the role of snakes as tertiary consumers found that they consumed 37 kg ha<sup>-1</sup> of prey annually (Willson and Winne 2015), which is far higher than typical consumption rates of avian or mammalian predators (Erlinge et al. 1983; Jedrzejewska and Jedrzejewska 1998). As a result, altered snake activity patterns (and consequent foraging behavior) in response to a changing climate could result in dramatic trophic cascades within ecosystems (DeGregorio et al. 2015). For any taxonomic group, how frequently an individual is active has significant implications for its ecology, as increased or decreased energy usage must be matched by a proportional shift in food intake (DeGregorio et al. 2015). Currently, only general information is available regarding how various environmental conditions might interact to influence snake activity (Daltry et al. 1998; Brown and Shine 2002; Abom et al. 2012; George et al. 2015), and often only at coarse temporal scales (i.e., seasonal activity patterns). Such studies also often involve highly laborious data collection schedules (i.e., 24 h tracking protocols) or automated telemetry techniques that also carry methodological limitations (i.e., snake proximity the automated receivers, physical barriers to signals, etc.) (Davis et al. 2008; Wasko and Sasa 2009; Abom et al. 2012; Howze and Smith 2012; Ward et al. 2013; Sperry et al. 2013; DeGregorio et

al. 2015). However, a common conclusion in these studies is that standard weather conditions at finer temporal scales often represent surprisingly poor predictors of activity, despite the seemingly clear line of inference between them (Brown and Shine 2002; Lindström et al. 2015). Complex interactions between internal and external states undoubtedly combine to drive variation in fine-scale activity decisions by individuals and teasing apart their relative roles is a challenging endeavor. Ultimately, a detailed evaluation of the relationships between animal behavior and the external environment (in wild-ranging individuals) requires consideration of patterns at multiple temporal scales, ranging from very short (i.e., daily) to extended periods (i.e., monthly, seasonal). The integrative RT-ACT framework validated in Chapter 2 offers a streamlined and easily transferrable approach for monitoring continuous activity and long-term spatial patterns over extended field deployments in snakes and other small, secretive terrestrial taxa, providing the foundation for future investigations using long-term RT-ACT monitoring to measure organismal responses to shifting environmental conditions.

Increased aridity is projected across the desert southwest of North America (Seager et al. 2007; Abatzoglou and Kolden 2011). In the case of the study system for the projects presented in Chapters 2 and 3, long-term data from Van Horn, Texas (32 km N of IMRS headquarters; Data source: NOAA-NCDC) show a significant increase in mean annual temperature and a decrease in total annual precipitation over the previous 25 years (Fig. 4.1A). Corresponding with this increasing aridity, a long-term mark-recapture population study on *C. atrox* at IMRS revealed significant annual variation and an overall decreasing trend in individual body condition from 2003 through 2015 (Fig 4.1B). Multiple comparisons (Tukey's HSD) revealed that recent years (2011, 2013, and 2014) had significantly lowered body condition, whereas 2007 and 2008 had significantly improved body condition. Given this correlation between physiology and aridity,

the stage is set for future investigations to establish the links between *C. atrox* activity, physiology, and increasing aridity, and the RT-ACT framework offers a “tailor-made” technique for such an undertaking. Despite being almost completely overlooked in this context, pitvipers, in general, represent ideal model organisms for investigating responses to contemporary and forecasted changes in abiotic conditions (Douglas et al. 2016).



**Figure 4.1** **A.** Time series (1990–2015) plot for Mean Annual Temperature (MAT) and Total Annual Precipitation (TAP) in Van Horn, Texas (ca. 32 km NE Indio Mountains Research Station). *P*-values derived from linear regression. **B.** Mean annual Body Condition Index (BCI) for all adult (SVL > 60 cm) *C. atrox* captured on IMRS from 2003–2015 (2006 not included because of logistical postponement of fieldwork; error bars  $\pm 1$  s.e.). Body condition was estimated with a Body Condition Index (BCI), which is the difference between the observed and predicted mass of an individual. Predicted masses were derived from a non-linear regression relating body mass to SVL for all *C. atrox* processed at IMRS: male mass (g) =  $1.51 \times 10^{-3} * \text{SVL (cm)}^{2.789}$ , female mass (g) =  $8.28 \times 10^{-2} * \text{SVL (cm)}^{1.858}$ . Numbers in parentheses indicate sample sizes ( $N = 244$ ). One-way ANOVA:  $F = 3.30$ ;  $P < 0.001$ ).

An emerging hallmark in the results provided by most animal bio-logging studies is the tremendous amount of variation between individuals, time periods (i.e., seasons), and populations (Rutz and Hays 2009). As evidenced in Chapter 2, this variation significantly limits statistical inference, particularly when paired with the cost-prohibitive nature of most loggers and often high logger failure rates (e.g., 7 of 19 deployed ACTs experienced premature battery failures) that contribute to limited sample sizes. As Hebblewhite and Haydon (2010) emphasized, more data does not always lead to an improved understanding. Furthermore, the “big data” provided by ACTs and other high-resolution sensors come at a considerable computational cost (as discussed in Chapter 1), even as a general set(s) of best practices slowly emerges in the literature (see Fig. 2.1). While these advanced technologies offer tremendous benefits relative to “primitive” techniques,

such as hand-held radio telemetry, it is important that ecologists thoroughly evaluate study goals before plunging into bio-logging applications, because the right tool for the job is highly context-dependent, and in many cases, traditional approaches still allow for strong study design (as seen in Chapter 3). It is also important to note that these challenges associated with bio-loggers will likely become less constraining as ongoing technological advances enable increasingly extensive field data collection periods from larger samples of animals. ACTs and other dataloggers will also become less cost-prohibitive as demand for these devices increases and more developers move into the animal bio-logging market-place. Despite the various challenges discussed here, when paired with a strong study design (long-term field monitoring and large samples), bio-logging frameworks can offer unprecedented explanatory power when inter- and intra-individual variation is placed in appropriate ecological or evolutionary contexts – such as establishing links between variability in individual behavior and physiology, individual performance, and population trajectories (Wilson et al. 2018) – an exciting avenue for future research with appreciable conservation implications.

Ultimately, for any animal, movement and activity are only a means to an end, as they generally represent efforts to locate and acquire resources (food, prey, water, mating partners, shelter, etc.) or avoid predators and competitors (Ropert-Coudert and Wilson 2005). Therefore, the critical next step in expanding the RT-ACT framework will be to incorporate additional cryptic behaviors in a refined classification scheme that can be used to establish links between the spatial and temporal dimensions of movement and the behavioral strategies or motivational states of individuals. In marine systems, this has been accomplished with great success in bio-logging research with pinnipeds, where interactions between dive frequency, dive duration, and food intake (measured via jaw movements detected with ACTs) are studied within an optimality framework



(Naito et al. 2010; Kienle et al. 2019; van Beest et al. 2019). With pitvipers, given extensive captive observations to obtain model training data, ACTs could feasibly be used to remotely measure feeding rates and reproductive behavior. At even finer scales, ACTs also have appreciable potential for quantifying the relationship between individual condition and performance in wild-ranging individuals, such as body temperature, strike kinematics, and predatory success rates. Using ACTs integrated with other bio-loggers to establish these previously elusive links now offers unprecedented potential for placing animal behavior in theoretical contexts (Watanabe et al. 2014; Wilmers et al. 2015; Westley et al. 2018).

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## Appendix

### **Ethics Statement:**

All methods were carried out in accordance with relevant guidelines and regulations, and all research protocols were approved for the entire study period by the University of Texas at El Paso Animal Care and Use Committee (Protocol A-201405-1) and Texas Parks and Wildlife (Scientific Permit Number SPR-0290-019).

### **Author initials appearing in-text:**

Dominic L. DeSantis (DLD)

### **R Code for RT-ACT Classification Models, Statistical Analyses, and Supplemental Figures:**

#### Chapter 2

#### **#Model Training and Testing with Random Forest and Elastic Net (GLMNET) Algorithms**

#this function preps the data for analysis

```
> prep.data=function(train)
> train$T=(as.POSIXlt((train$Time),format="%H:%M:%S"))
> train$H=hour(train$T)
> train$Date2=(as.POSIXlt((train$Date),format="%m/%d/%Y"))
> train$D=month(train$Date2)
```

```

> train$Diel[train$T >= as.POSIXct("06:00", format = "%H:%M") & train$T <
as.POSIXct("09:59", format = "%H:%M")]<-"morning"

> train$Diel[train$T >= as.POSIXct("10:00", format = "%H:%M") & train$T <
as.POSIXct("17:59", format = "%H:%M")]<-"diurnal"

> train$Diel[train$T >= as.POSIXct("18:00", format = "%H:%M") & train$T <
as.POSIXct("20:59", format = "%H:%M")]<-"evening"

> train$Diel[train$T >= as.POSIXct("21:00", format = "%H:%M") | train$T <
as.POSIXct("05:59", format = "%H:%M")]<-"nocturnal"

> train[,c("IX")]=log(train[,c("X")]+1-min(train[,c("X")],na.rm=TRUE))

> train[,c("IY")]=log(train[,c("Y")]+1-min(train[,c("Y")],na.rm=TRUE))

> train[,c("IZ")]=log(train[,c("Z")]+1-min(train[,c("Z")],na.rm=TRUE))

> train$x1=c(NA,diff(train$IX))

> train$y1=c(NA,diff(train$IY))

> train$z1=c(NA,diff(train$IZ))

> train$x10=c(rep(NA,20),diff(train$IX,lag=20))

> train$y10=c(rep(NA,20),diff(train$IY,lag=20))

> train$z10=c(rep(NA,20),diff(train$IZ,lag=20))

> odba=function(x) {return(abs(x[1])+abs(x[2]))}

> vdba=function(x) {return(sqrt(x[1]^2+x[2]^2))}

> train$ODBA=apply(train[,c("IX","IY","IZ")],1,odba)

> train$VDBA=apply(train[,c("IX","IY","IZ")],1,vdba)

> train$sdX=c(rep(NA,19),rollapply(train[,c("IX")],width=20,FUN=sd))

> train$meanX=c(rep(NA,19),rollapply(train[,c("IX")],width=20,FUN=mean))

```

```

> train$minX=c(rep(NA,19),rollapply(train[,c("IX")],width=20,FUN=min))
> train$maxX=c(rep(NA,19),rollapply(train[,c("IX")],width=20,FUN=max))
> train$slopeX=c(NA,diff(train$meanX))
> train$sdY=c(rep(NA,19),rollapply(train[,c("IY")],width=20,FUN=sd))
> train$meanY=c(rep(NA,19),rollapply(train[,c("IY")],width=20,FUN=mean))
> train$minY=c(rep(NA,19),rollapply(train[,c("IY")],width=20,FUN=min))
> train$maxY=c(rep(NA,19),rollapply(train[,c("IY")],width=20,FUN=max))
> train$slopeY=c(NA,diff(train$meanY))
> train$sdZ=c(rep(NA,19),rollapply(train[,c("IZ")],width=20,FUN=sd))
> train$meanZ=c(rep(NA,19),rollapply(train[,c("IZ")],width=20,FUN=mean))
> train$minZ=c(rep(NA,19),rollapply(train[,c("IZ")],width=20,FUN=min))
> train$maxZ=c(rep(NA,19),rollapply(train[,c("IZ")],width=20,FUN=max))
> train$slopeZ=c(NA,diff(train$meanZ))
> ps=function(x){x-median(x)}
> st=function(x){x-mean(x)}
> train$Xps=ps(train[,4])
> train$Yps=ps(train[,5])
> train$Zps=ps(train[,6])
> train$Xst=st(train[,4])
> train$Yst=st(train[,5])
> train$Zst=st(train[,6])
> train$Xps2=ps(train[train$TagID==levels(train$TagID)[1],4])
> train$Yps2=ps(train[train$TagID==levels(train$TagID)[1],5])

```



```

>train$Zps2=ps(train[train$TagID==levels(train$TagID)[1],6])
> train$Xst2=st(train[train$TagID==levels(train$TagID)[1],4])
>train$Yst2=st(train[train$TagID==levels(train$TagID)[1],5])
>train$Zst2=st(train[train$TagID==levels(train$TagID)[1],6]) return(train)}
> readin data head(train)
> train=train[,-c(10,11)]
> colnames(train)=c("TagID","Date","Time","X","Y","Z","Pressure","Body.Temp","Behavior")
> train[,c("X","Y","Z")]=scale(train[,c("X","Y","Z")])
> train$D=month(as.Date(train$Date,format = "%m/%d/%y"))
> train$T=as.POSIXct(as.character(train$Time),format="%H:%M:%S")
> train$Diel[train$T >= as.POSIXct("06:00", format = "%H:%M") & train$T <
as.POSIXct("09:59", format = "%H:%M")]<-"morning"
> train$Diel[train$T >= as.POSIXct("10:00", format = "%H:%M") & train$T <
as.POSIXct("17:59", format = "%H:%M")]<-"diurnal"
> train$Diel[train$T >= as.POSIXct("18:00", format = "%H:%M") & train$T <
as.POSIXct("20:59", format = "%H:%M")]<-"evening"
> train$Diel[train$T >= as.POSIXct("21:00", format = "%H:%M") | train$T <
as.POSIXct("05:59", format = "%H:%M")]<-"nocturnal"
> train=prep.data(train)
> dim(train)
> train$Behavior[train$Behavior=="Not moving "]= "Not Moving"
> train$Behavior[train$Behavior=="Not Moving "]= "Not Moving"
> train$Behavior=factor(train$Behavior,levels=c("Moving","Not Moving"))

```

```

> head(train)

#create test data

> samp=sample(1:nrow(train),round(nrow(set2)/3))

> test=na.omit(train[samp,])

> train2=na.omit(train[-samp,])

> saveRDS(fullt,"fullrds.txt")

> fullt=fread("fullt.txt")

> fullt$Day=day(fullt$Date2)

> head(fullt)

> class(fullt)

> fwrite(fullt,"fullf.csv")

> table(fullt$D,fullt$TagID)

> fullt[,11:36]=apply(full[,11:36],2,scale)

#trained model makes predictions

> head(fullt)

> table(train$Behavior)

```

### **#Random Forest (RF) Model**

```

> mod=randomForest(Behavior~lX+lY+lZ+sdX+meanX+minX+maxX+slopeX+sdY+meanY+m
inY+maxY+slopeY+sdZ+VDBA,ntree = 1000, data=train2)

```

```

>p.test=predict(mod,test[,c("x1","y1","z1","lX","lY","lZ","sdX","meanX","minX","maxX","slopeX","sdY","meanY","minY","maxY","slopeY","sdZ","meanZ","minZ","maxZ","slopeZ","ODBA","VDBA")])

> prop.table(table(p.test,test$Behavior),2)

> table(p.test,test$Behavior)

>p.rf=predict(mod,fullt[,c("x1","y1","z1","lX","lY","lZ","sdX","meanX","minX","maxX","slopeX","sdY","meanY","minY","maxY","slopeY","sdZ","meanZ","minZ","maxZ","slopeZ","ODBA","VDBA")])

> table(p.rf)

> png("varimp.png",units="px",width=1600,height=1600,res=300)

> varImpPlot(mod,type=2)

> dev.off()

#reformat predictions

> t.5<-data.frame(Behavior=p.rf[fullt$D==5],Diel=fullt$Diel[fullt$D==5])

> t.6<-data.frame(Behavior=p.rf[fullt$D==6],Diel=fullt$Diel[fullt$D==6])

> t.8<-data.frame(Behavior=p.rf[fullt$D==8],Diel=fullt$Diel[fullt$D==8])

> t.9<-data.frame(Behavior=p.rf[fullt$D==9],Diel=fullt$Diel[fullt$D==9])

> t.10<-data.frame(Behavior=p.rf[fullt$D==10],Diel=fullt$Diel[fullt$D==10])

```

### **#GLMM-Elastic Net Model**

```

> alphas <- seq(0,1,by=0.2)

#overall model

```

```

>elasticnet<-lapply(alphas,function(a){(mod<-cv.glmnet(as.matrix(train[-
(1:5),c("x1","y1","z1","lX","lY","lZ","sdX","meanX","minX","maxX","slopeX","sdY","meanY"
,"minY","maxY","slopeY","sdZ","meanZ","minZ","maxZ","slopeZ","ODBA","VDBA"))],nrow=
dim(set2)[1]),y=as.factor(train$Behavior[-(1:5)]),type.measure="auc",
family="binomial",nfolds=10,alpha=alphas));print(min(mod$cvm))})

>mod=cv.glmnet(as.matrix(train[-
(1:5),c("x1","y1","z1","lX","lY","lZ","sdX","meanX","minX","maxX","slopeX","sdY","meanY"
,"minY","maxY","slopeY","sdZ","meanZ","minZ","maxZ","slopeZ","ODBA","VDBA"))],nrow=
dim(set2)[1]),y=as.factor(train$Behavior[-(1:5)]),type.measure="auc",
family="binomial",nfolds=10,alpha=alphas[which(elasticnet==min(unlist(elasticnet)))],keep=TR
UE)

> coef(mod,s="lambda.min")

>p.testg=predict(mod,newx=data.matrix(test[,c("x1","y1","z1","lX","lY","lZ","sdX","meanX","
minX","maxX","slopeX","sdY","meanY","minY","maxY","slopeY","sdZ","meanZ","minZ","m
axZ","slopeZ","ODBA","VDBA"))],s="lambda.min",type="class")

#make predictions

> prop.table(table(p.testg,test$Behavior),2)

> table(p.testg,test$Behavior)

```

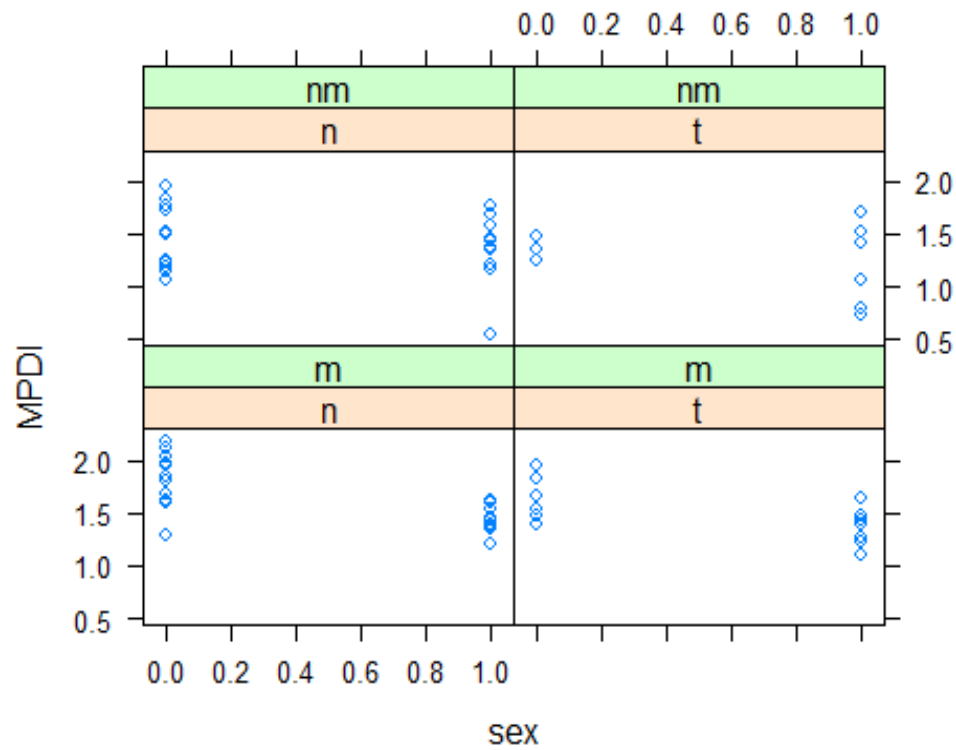
### Chapter 3

**#Generalized Linear Mixed Effects Models (GLMMs) for movement and space Use metrics with Least Squared (LS) means contrasts for pairwise comparisons**

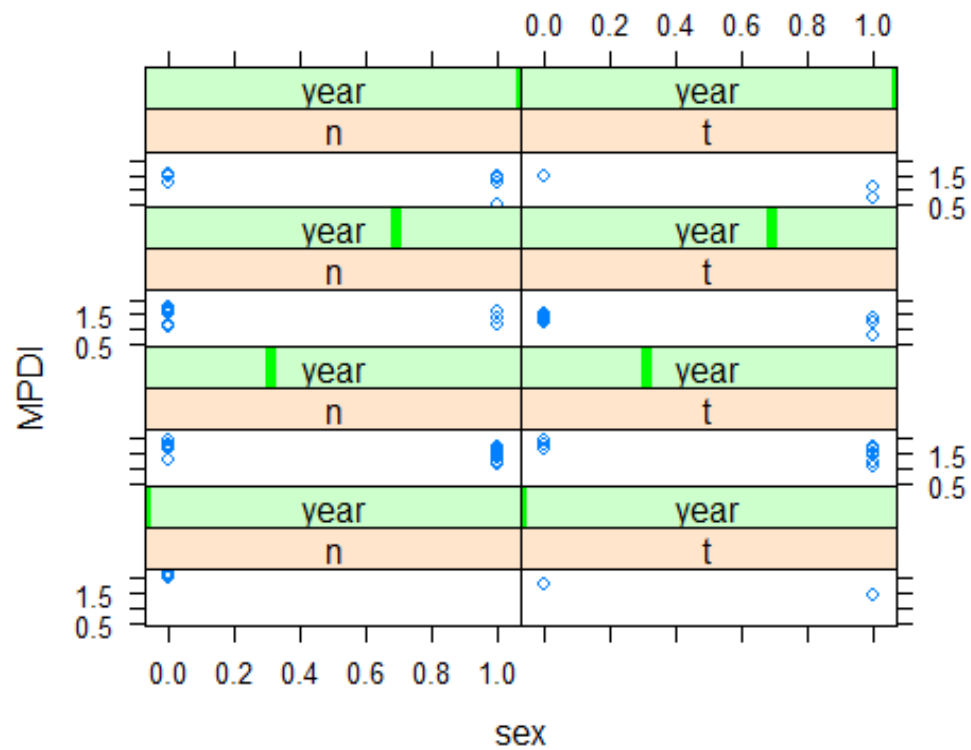
#plot showing differences in spread and location of MPD across sex and within habitat and season

```
> dat$MPDI=log10(dat$MPD)
```

```
> xyplot(MPDI~sex|habitat*season,data=dat)
```

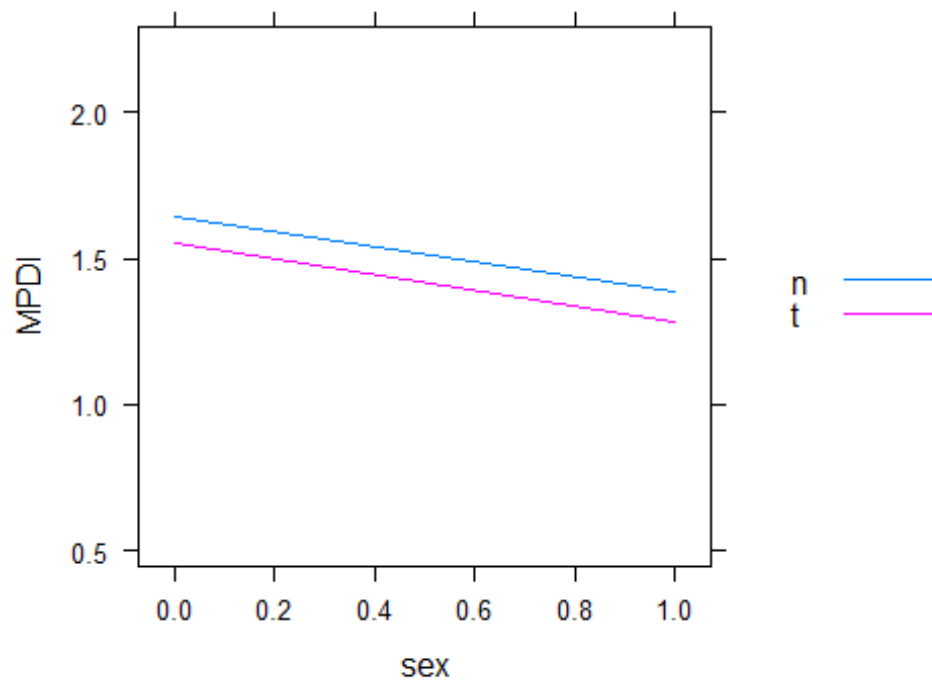


```
> xyplot(MPDI~sex|habitat*year,data=dat)
```

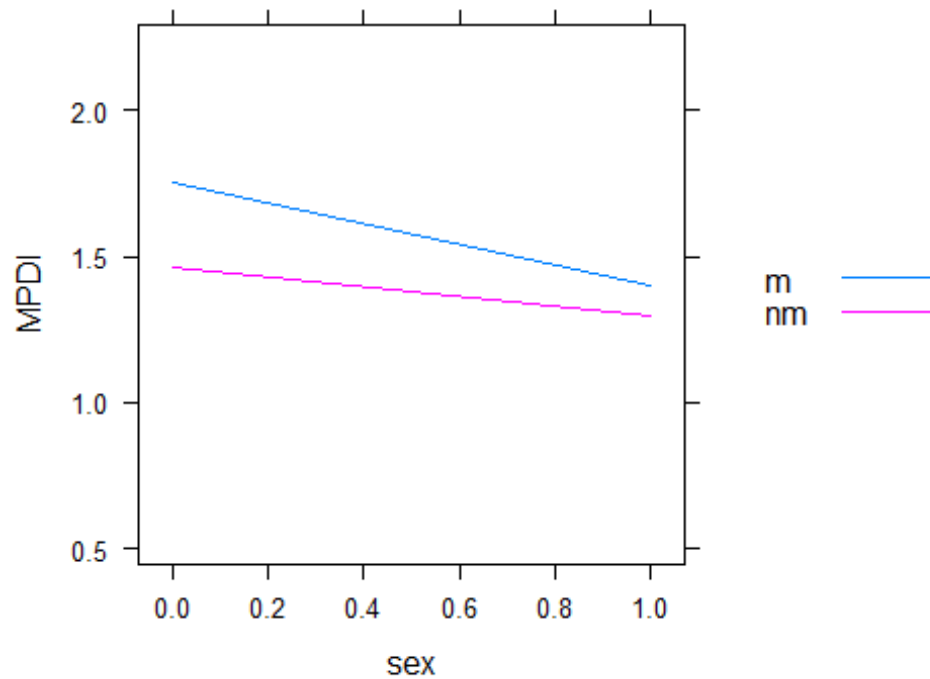


#plot showing differences in MPDI across sex with habitat and season interaction (separately)

```
> xyplot(MPDI ~ sex, dat, groups = habitat, type = "a", auto.key = list(space = "right", points = FALSE, lines = TRUE))
```

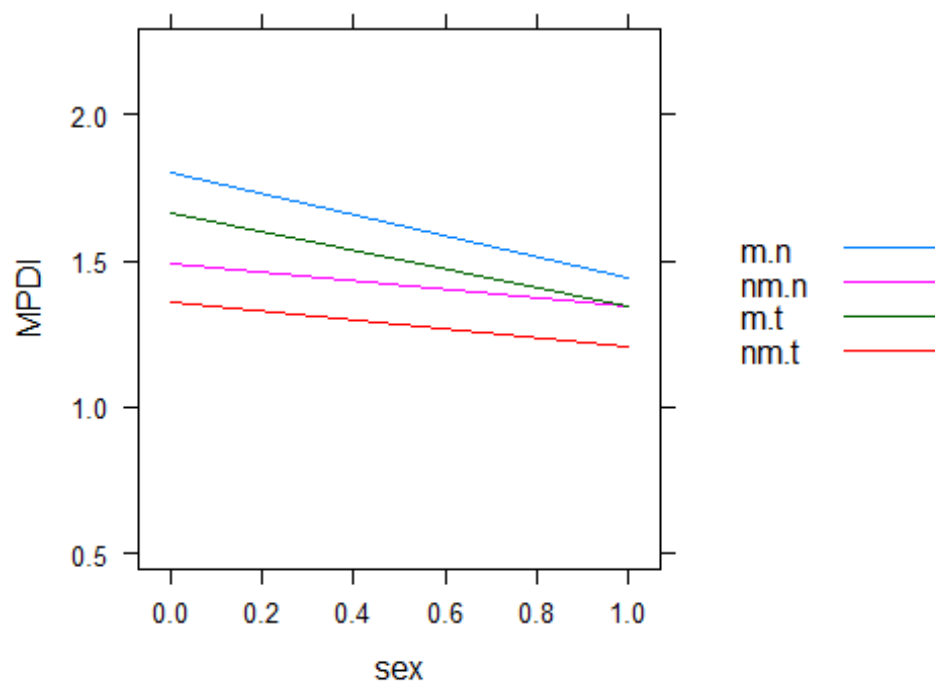


```
> xyplot(MPDI ~ sex, dat, groups = season, type = "a", auto.key = list(space = "right", points = FALSE, lines = TRUE))
```



```
> dat$SeaHab=interaction(dat$season,dat$habitat)
```

```
> xyplot(MPDI ~ sex, dat, groups = SeaHab, type = "a", auto.key = list(space = "right", points = FALSE, lines = TRUE))
```





```
#ggplot(dat,aes(sex,MPDl,season,habitat,ID))+geom_point(dat,aes(sex,MPDl,season,habitat,ID)))

> mpdmodel <- lmer(MPDl ~ as.factor(sex)*season*habitat+(1|ID)+(1|ID:year),data=dat)

> summary(mpdmodel)

> lsmeans(mpdmodel,pairwise~"sex")

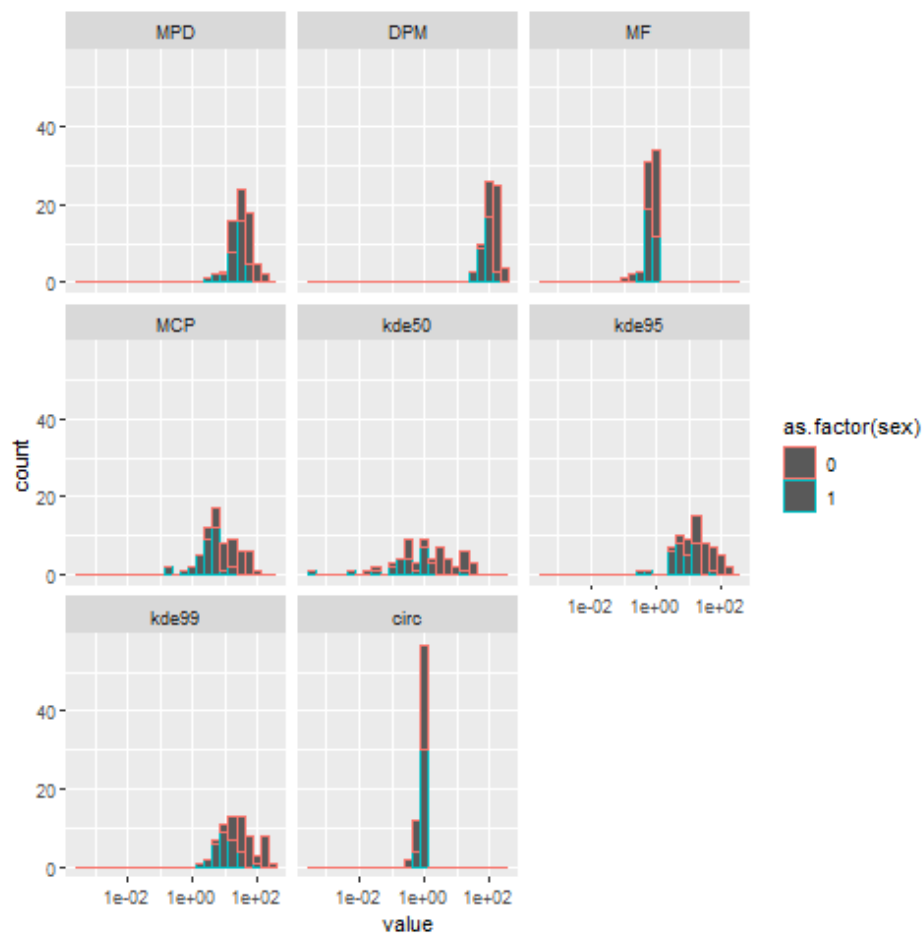
> lsmeans(mpdmodel,pairwise~season|sex)

> lsmeans(mpdmodel,pairwise~season|sex*habitat)

> lsmeans(mpdmodel,pairwise~habitat|sex*season)

> r.squaredGLMM(mpdmodel)

#Combined histogram (sex as factor) for movement and space use metrics
```



#additional movement and space use models (not displayed here) used same source code

### **#GLMMs for Reproductive Behavior**

```
> dat$SVLl=log10(dat$SVL)
```

```
> dat$MPDl=log10(dat$MPD)
```

```
> dat$DPMl=log10(dat$DPM)
```

```
> dat$MCPl=log10(dat$MCP)
```

```
> dat$KDE50l=log10(dat$KDE50)
```

```
> dat$KDE95l=log10(dat$KDE95)
```

```
> dat$MMF1=logit(dat$MMF,adjust=TRUE)
```

```
> dat$dir1=logit(dat$Directionality,adjust=TRUE)
```

```
> dat$Sexf=factor(dat$Sex)
```

```
> dat$Habf=factor(dat$Habitat)
```

```
> dats=data.frame(scale(dat[,c(2,5:11)]),dat[,~c(2,5:11)])
```

```
#hist(dats$partners)
```

```
> dat$partl=log10(dat$partners+1)
```

```
#summary(dats$partl)
```

```
> pmod0=glmer(partners~(Habf)+(SVLl)+Sexf+(MPDl)+(DPMl)+(MMF1)+(MCP1)+(KDE50l)+  
(KDE95l)+(dir1)+(1|ID),data=(dat),family=poisson(link="log"))
```

```
> summary(pmod0)
```

```
#lsmeans(pmod0,pairwise~"Sexf")
```

```
> plot(pmod0, which=c("Picture", "TVset"), pairwise = TRUE)
```

```
> pmod1=glmer(partners~(Habf)+(SVLl)+Sexf+(MPDl)+(DPMl)+(MMF1)+(MCP1)+(KDE50l)+  
(KDE95l)+(dir1)+(1|ID)+(1|Year),data=dat,family=poisson(link="log"))
```

```
#family=poisson(link="log")
```

```

> summary(pmod1)

> AIC(pmod0,pmod1)

> BIC(pmod0,pmod1)

> mod1=lmerTest::lmer(pmod0,data=dat)

> summary(mod1)

> ci.res=confint.merMod(pmod0,method="Wald")[-c(1:2),]

> eff=cbind(summary(mod1)$coefficients[,4],summary(mod1)$coefficients[,3],summary(mod1)$
> coefficients[,5])

> colnames(eff)<-c("t","df","pval")

> eff<-as.data.frame(eff[-1,])

> eff$d=(2*eff$t)/sqrt(eff$df) (temp=data.frame(eff$t,eff$d,eff$df,eff$pval,ci.res))

#compare means

> confint(lsmmeans(mod1.1,pairwise~HS))

>
modt=lmer(partners~0+(Habf+Sexf)*MPDl+SVLl+DPMl+MMFl+MCPi+KDE50l+KDE95l+dir
l+(1|ID),data=dat)

> confint(lsmmeans(modt,pairwise~Habf|MPDl))

> confint(lsmmeans(modt,pairwise~Sexf|MPDl))

>modt=lmer(partners~0+(Habf+Sexf)*SVLl+MPDl+DPMl+MMFl+MCPi+KDE50l+KDE95l+d
irl+(1|ID),data=dat)

> confint(lsmmeans(modt,pairwise~Habf|SVLl))

> confint(lsmmeans(modt,pairwise~Sexf|SVLl))

>modt=lmer(partners~0+(Habf+Sexf)*DPMl+SVLl+MPDl+MMFl+MCPi+KDE50l+KDE95l+d
irl+(1|ID),data=dat)

> confint(lsmmeans(modt,pairwise~Habf|DPMl))

```

```

> confint(lsmmeans(modt,pairwise~Sexf|DPMI))

>modt=lmer(partners~0+(Habf+Sexf)*MMFI+SVLI+MPDI+DPMI+MCPi+KDE50I+KDE95I+d
irl+(1|ID),data=dat)

> confint(lsmmeans(modt,pairwise~Habf|MMFI))

> confint(lsmmeans(modt,pairwise~Sexf|MMFI))

>modt=lmer(partners~0+(Habf+Sexf)*MCPi+SVLI+MPDI+DPMI+MMFI+MCPi+KDE50I+KD
E95I+dirl+(1|ID),data=dat)

> confint(lsmmeans(modt,pairwise~Habf|MCPi))

> confint(lsmmeans(modt,pairwise~Sexf|MCPi))

>modt=lmer(partners~0+(Habf+Sexf)*KDE50I+SVLI+MPDI+DPMI+MMFI+MCPi+KDE95I+d
irl+(1|ID),data=dat)

> confint(lsmmeans(modt,pairwise~Habf|KDE50I))

> confint(lsmmeans(modt,pairwise~Sexf|KDE50I))

>modt=lmer(partners~0+(Habf+Sexf)*KDE95I+SVLI+MPDI+DPMI+MMFI+MCPi+KDE50I+d
irl+(1|ID),data=dat)

> confint(lsmmeans(modt,pairwise~Habf|KDE95I))

> confint(lsmmeans(modt,pairwise~Sexf|KDE95I))

>modt=lmer(partners~0+(Habf+Sexf)*dirl+SVLI+MPDI+DPMI+MMFI+MCPi+KDE50I+KDE
95I+(1|ID),data=dat)

> confint(lsmmeans(modt,pairwise~Habf|dirl))

> confint(lsmmeans(modt,pairwise~Sexf|dirl))

#attendance-days-per-partner model (not displayed here) used same source code

#Index of Dispersion: k-nearest neighbor calculations at weekly intervals

> dat$xn=scale(dat$x)

> dat$yn=scale(dat$y)

> names(dat)=c("id","week","x","y","xn","yn")

```

```
> summary(dat)

> table((dat$week))

#using Euclidean distance

> for (j in levels(dat$week)){E.dist=dist(dat[dat$week==j,5:6], method = "euclidean", diag =
FALSE, upper = FALSE, p = 2) res=na.omit(E.dist[lower.tri(E.dist)])

print(paste(j,"=",mean(res)/var(res)))
```

**Image of *Crotalus atrox* subjected to RT-ACT monitoring on Indio Mountains Research Station:**



## **Vita**

Dominic L. DeSantis earned his B.S. in Wildlife Biology from Texas State University in December 2013 and began his Ph.D. at UTEP in August 2014. While at UTEP, he was an assistant instructor for the department of biological sciences during the 2014–2016 and 2017–2018 academic years, a National Science Foundation-Graduate Research Fellow from 2016–2019, and a research assistant for the department for biological sciences from 2016–2019. Dominic's research interests broadly include the behavioral ecology, conservation biology, and natural history of herpetofauna (reptiles and amphibians), and he has accumulated over 50 peer-reviewed publications (12 major papers, 44 natural history notes) on topics within these fields. He has also delivered (12) or co-authored (7) 19 professional presentations at local and international meetings. As a doctoral student, Dominic was awarded the George A. Krutilek Memorial Graduate Fellowship (2015), Graduate Student Summer Research Grant (2015), Dodson Research Grant (2015, 2016), Outstanding Doctoral Student Award (2016), Frank B. Cotton Trust Scholarship (2016–2018), Graduate Fellowship Incentive Award (2016, 2019), and the NSF-Graduate Research Fellowship (2016–2019). Dominic also actively engaged in undergraduate student mentorship while at UTEP through NSF-Research Experience for Undergraduates and UTEP-Campus Office for Undergraduate Involvement in Research programs. He closely mentored nine undergraduates, all of whom presented findings from their projects at the UTEP Undergraduate Research Symposium. Dominic intends to pursue a post-doctoral position to expand upon the novel data collection, processing, and analysis techniques developed during his dissertation. In addition to his academic pursuits, Dominic is a proud husband and father that seeks out every opportunity to enjoy the outdoors with his family.

This dissertation was typed by Dominic Louis DeSantis.